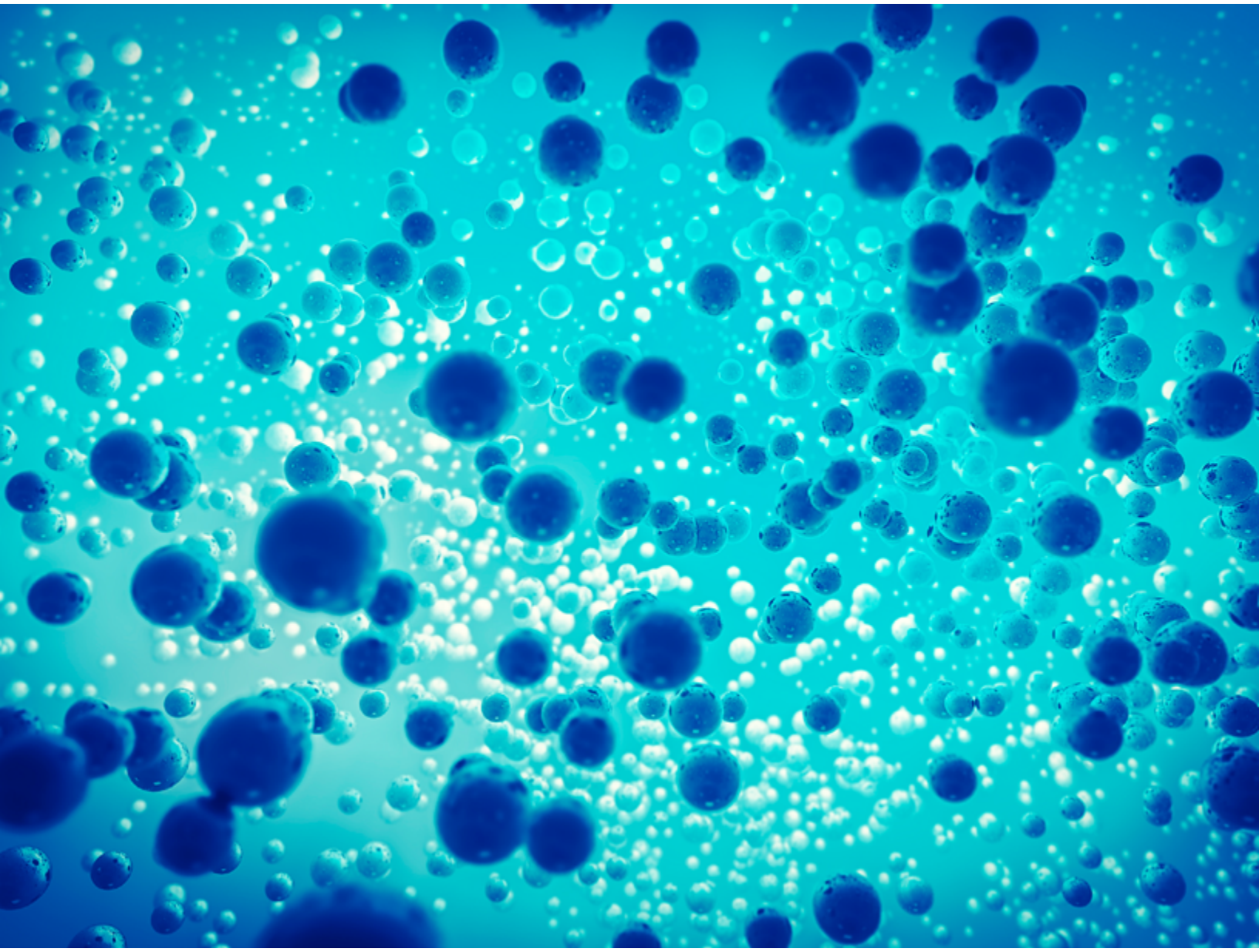
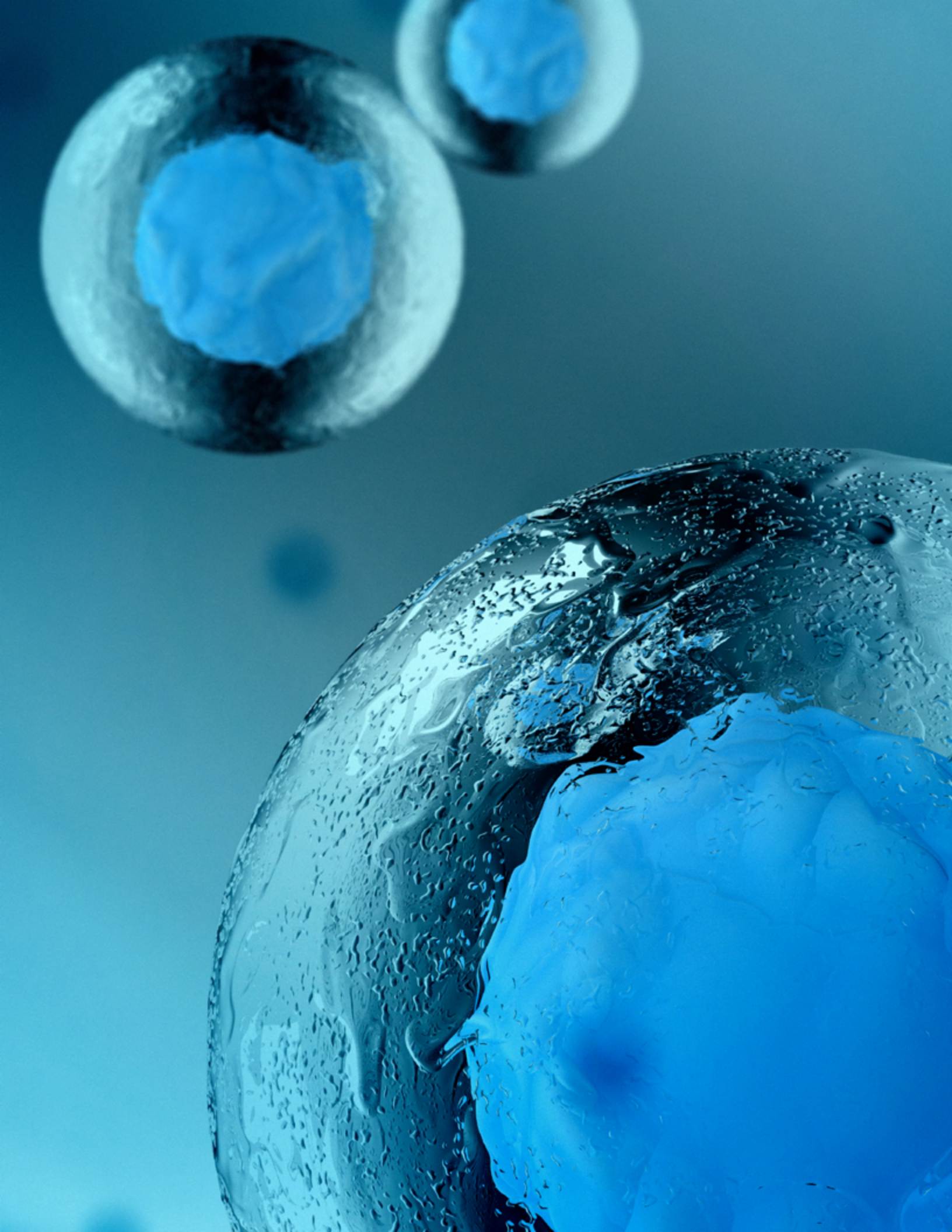
10x GENOMICS

Demystifying single cell sequencing

Get to know the technology that is changing how we do science





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Introduction

Bringing the moonshot within reach

Every field of biology has a moonshot vision. Identify a new cell type in a poorly characterized tissue. Fully characterize the complex factors that control infection severity. Find a lasting, effective drug target for Alzheimer's disease. Reach 100% remission rates for immunotherapy across patients and cancer types. From basic discovery to translational applications, these are the research dreams that inspire relentless curiosity and persistent progress.

What will ultimately drive this progress is the collaborative science of everyday researchers. Discoveries build upon one another, adding to an increasingly clear picture of biological complexity, and taking the field one step closer to its moonshot.

"Genes and gene products do not function independently, but participate in complex, interconnected pathways, networks and molecular systems that, taken together, give rise to the workings of cells, tissues, organs and organisms. Defining these systems and determining their properties and interactions is crucial to understanding how biological systems function."

- Collins F, et al. A vision for the future of genomics research. Nature 422: 835–847 (2003). (1)

In order to advance discovery, scientists need tools that can provide a comprehensive view of the underlying biological complexity of their samples. For a growing number of researchers, single cell RNA-sequencing and single cell multiomics—genomic approaches for quantitative analysis of gene expression and other DNA, RNA, or protein-based analytes at single cell resolution—are proving effective to address their biological questions. Enabling investigation of biology in its smallest functional unit, these tools clarify the details to ultimately build a bigger, more nuanced picture of the interactions between different types of molecules and cells that drive an external phenotype: why do cancer patients relapse? What made this vaccine effective to hold off infection?

As you consider the contributions you want to make in your field—how you hope to move studies forward in meaningful ways, taking steps to reach that moonshot—it will be essential to equip yourself with tools that can help you reach your goals. Single cell studies have proven their value in publication form since 2009 (2), with applications and scope building over time as each field sees what can be done and notes the promise it holds for their own work. This eBook will guide you through your introduction to single cell sequencing technology, including how it works, how it parallels techniques you are already familiar with, and the new depth of insight it can provide for your biological questions. We will address key differences from your current processes as well as many research examples that can serve to prepare you and give you confidence in your ability to succeed with single cell sequencing. Finally, we will provide helpful resources to walk you through the next steps of your single cell journey.

References

1. Collins F, et al. A vision for the future of genomics research. *Nature* 422: 835–847 (2003).
2. Aldridge S and Teichmann SA. Single cell transcriptomics comes of age. *Nat Commun* 11: 4307 (2020).

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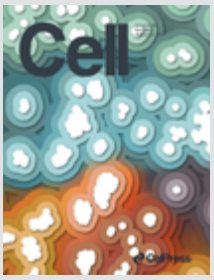
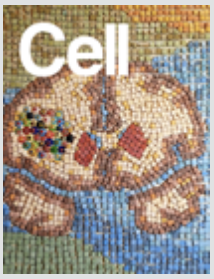
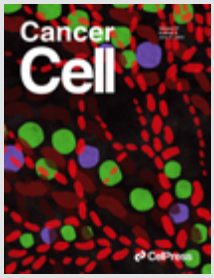
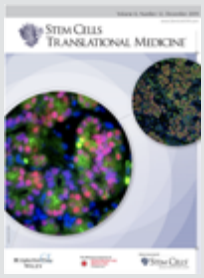
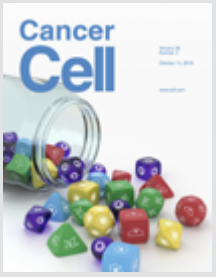
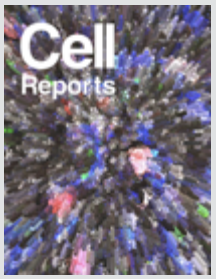


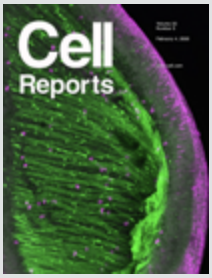












Cover images from top journals depict research using 10x Genomics single cell technology.

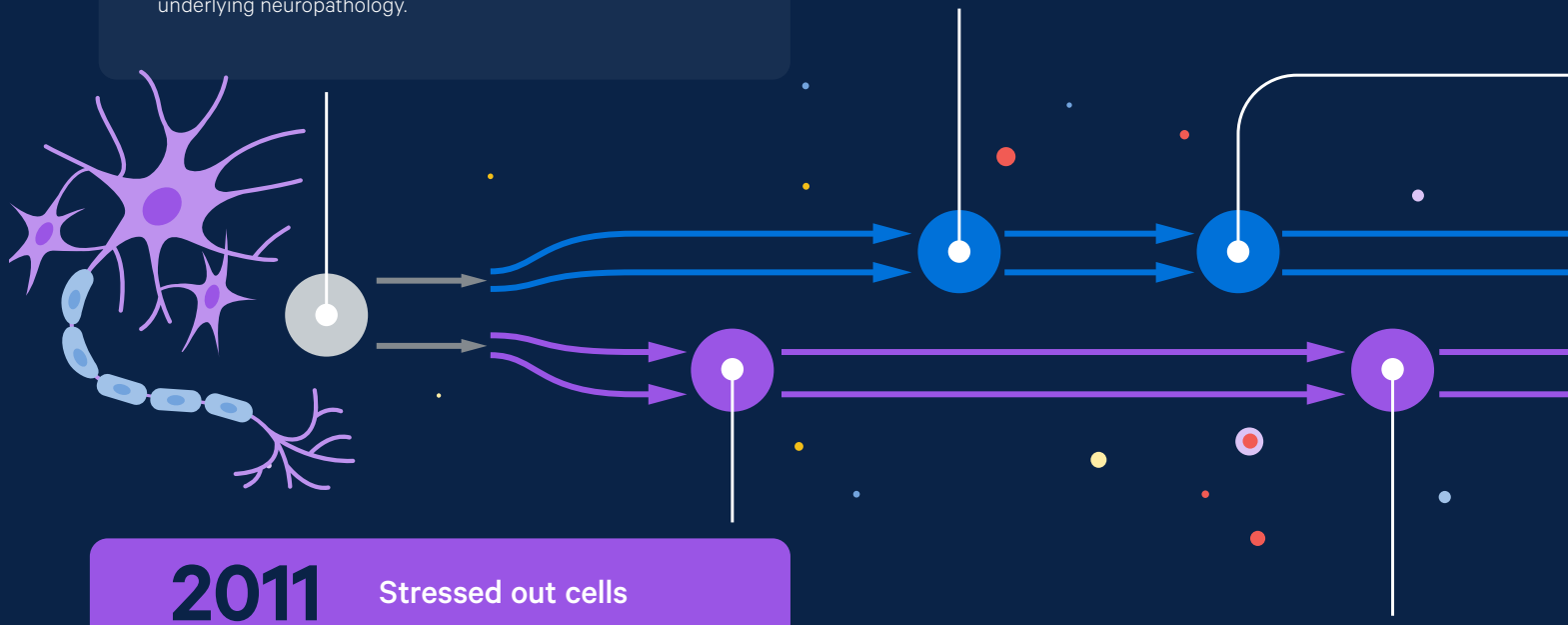
Paving new roads of discovery for Alzheimer's disease research

1990s What we didn't know about Alzheimer's

- We didn't know the ways in which **microglia**, the immune sentinels of the central nervous system, are phenotypically responsive to their environment and that they play a crucial role in Alzheimer's disease (AD) pathology.
- We didn't know why **some neurons are more vulnerable** than others to AD-related tau and Aß pathology, leading to diverse clinical manifestations of the disease despite a common, broad underlying neuropathology.

2014 More than M1 and M2

- Traditionally classified into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes, scientists confirm that microglia do not fit a dual classification system, but likely appear as a continuum of cell states in response to environmental triggers, including pathogens and damage (1).



2011 Stressed out cells

- Scientists postulate that intrinsic and environment-induced cellular stress and homeostasis pathways may contribute in parallel to the accumulation of misfolding proteins in particular vulnerable neurons to promote neurodegenerative disease (4).

2019 Excitatory neurons at risk

- Excitatory neurons are shown to have greater vulnerability to AD-related tauopathy than inhibitory neurons (5).

References:

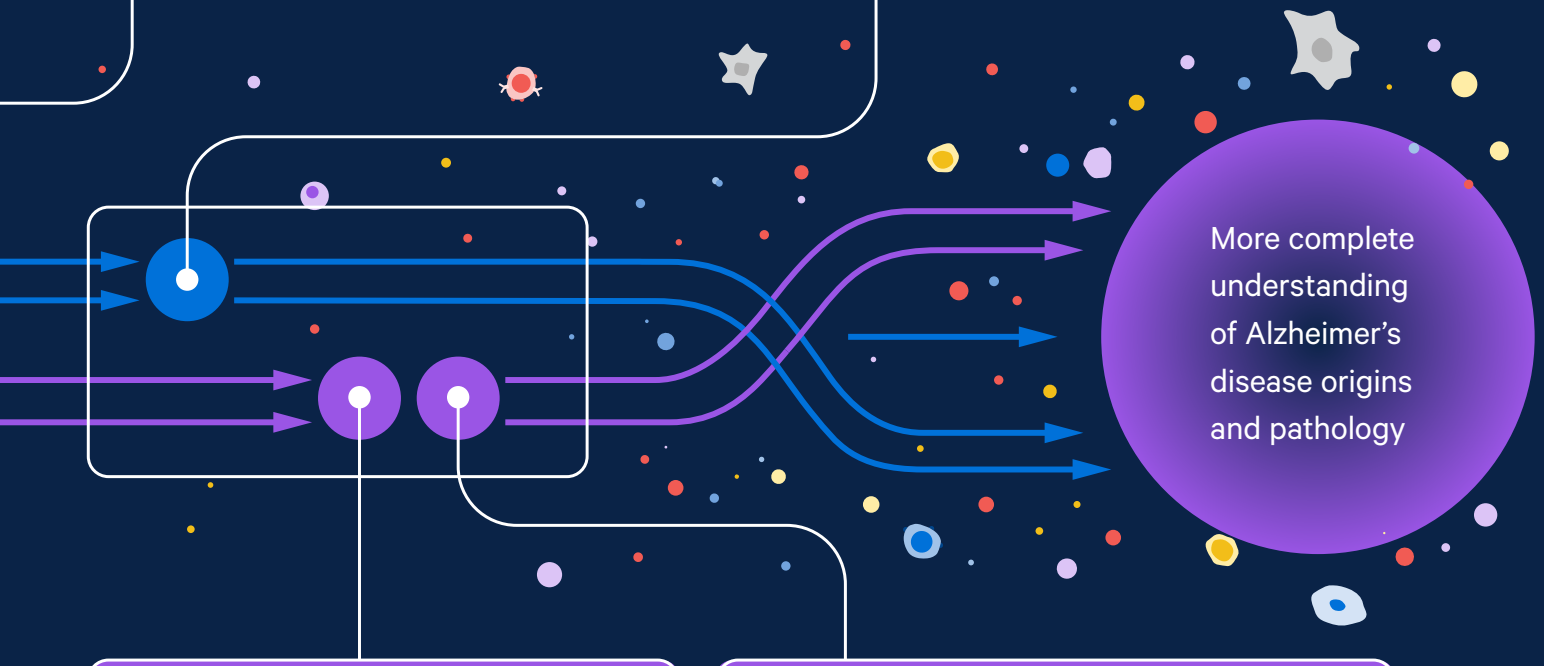
1. Gertig U and Hanisch UK. Microglial diversity by responses and responders. *Front Cell Neurosci* 8: 101 (2014).
2. Rangaraju S, et al. Identification and therapeutic modulation of a pro-inflammatory subset of disease-associated-microglia in Alzheimer's disease. *Mol Neurodegener* 13: 24 (2018).
3. Olah M, et al. Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Comm* 11, 6129 (2020).
4. Saxena S and Caroni P. Selective Neuronal Vulnerability in Neurodegenerative Diseases: from Stressor Thresholds to Degeneration. *Neuron* 71: 35–48 (2011).
5. Fu H, et al. A tau homeostasis signature is linked with the cellular and regional vulnerability of excitatory neurons to tau pathology. *Nat Neurosci* 22: 47–56 (2019).
6. Leng K, et al. Molecular characterization of selectively vulnerable neurons in Alzheimer's disease. *Nat Neurosci* 24: 276–287 (2021).
7. Zalocusky KA, et al. Neuronal ApoE upregulates MHC-I expression to drive selective neurodegeneration in Alzheimer's disease. *Nat Neurosci* 24: 786–798 (2021).

2018 Discovering the DAMs

- A microglial subpopulation called disease-associated microglia (DAM) phenotype are classified in neurodegenerative disease, chronic neuroinflammatory states, and aging (2).

2020 The missing microglia

- Single cell transcriptomic data reveals an Aß-plaque-associated microglial subtype bearing a disease-associated gene expression signature with reduced frequency in AD samples (3).
- **Read more**



2021 Markers of vulnerability across the brain

- Transcriptional profiling reveals a select set of genes expressed by several subpopulations of vulnerable neurons, including RAR-related Orphan Receptor B, across brain regions (6).
- **Read more**

2021 A new putative target to prevent selective neurodegeneration

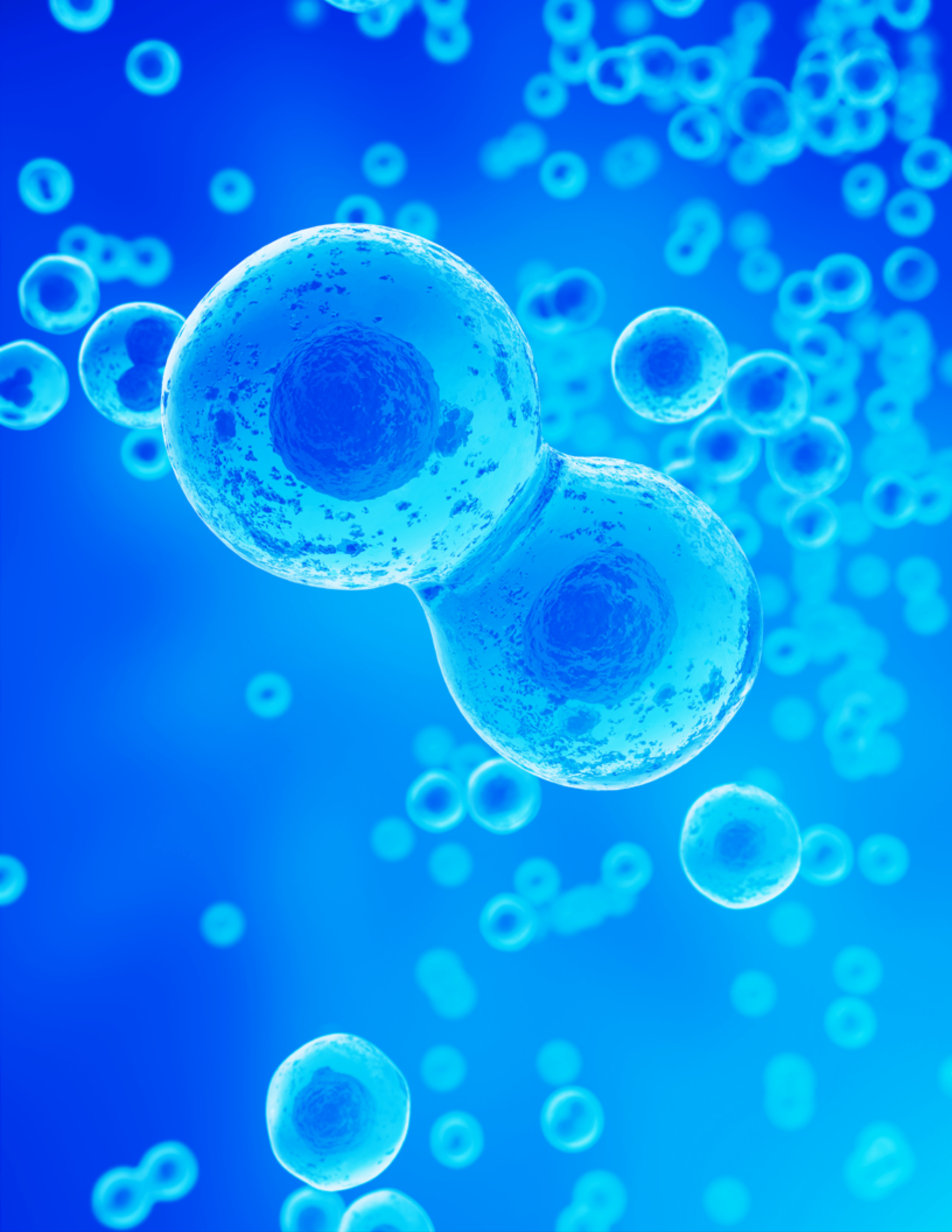
- Neuronal ApoE expression is shown to drive MHC-1 gene expression, potentially marking neurons for destruction by immune effector cells and leading to an increased loss of vulnerable neurons (7).
- **Read more**

Microglia activity

Selective neuronal vulnerability

Enabled by 10x Genomics single cell technology

Figure 1. Chronicling advances in our understanding of Alzheimer's disease. Leading neuroscientists are using 10x Genomics technology in their game-changing endeavors, paving new roads with collaboration and innovation as they strive to master the complexities of Alzheimer's disease pathogenesis.



Chapter 1

Why single cell sequencing should matter to you

Enabling high-impact science

You are a scientist, in search of answers to the most important questions in health and disease. You're a neuroscientist looking to understand the healthy cellular processes that are dysregulated in neurodevelopmental disease. You're an immunologist searching for the gene expression signatures and biomarkers that define an inflammatory condition. You're a cancer researcher trying to uncover the intratumoral heterogeneity that confounds therapies.

Bringing these answers into focus requires innovative experimentation and technological capabilities that continuously push the boundaries of what's possible—because discoveries are made at the leading edge of technology. A telescope with two lenses allowed Italian astronomer Galileo Galilei to observe craters in the moon. But a redesigned telescope with higher quality lenses and a longer shaft magnified his vision eightfold—then, through further refinement, thirtyfold, bringing the planet Jupiter and three of its orbiting moons into clear view (1).

Why single cell resolution?

Biology is like the universe. It is highly complex, with many unique singular bodies—cells—interacting with one another and playing different roles to build and drive processes within a larger system. Like Galileo's efforts to explore the universe, discovering and defining these cells requires high-resolution tools able to untangle the gene expression heterogeneity that contributes to biological complexity.

Bulk transcriptomic approaches like RNA sequencing (RNA-seq) or microarray analysis represent a powerful class of tools that helped scientists begin to unravel this heterogeneity, providing an average view of gene expression from a mixed sample. Subsequent leaps in technological innovation have culminated in single cell approaches that allow scientists to define cell type–specific gene expression, resolving the cellular heterogeneity that drives the expression patterns seen from an average readout (Figure 2). This gives researchers the ability to more fully characterize tissue heterogeneity, identify rare cell types, and dissect molecular mechanisms cell by cell. With a truer picture of their biological systems, researchers have a confident foundation of knowledge from which they can plan their next experiments and generate deeper, actionable insights.

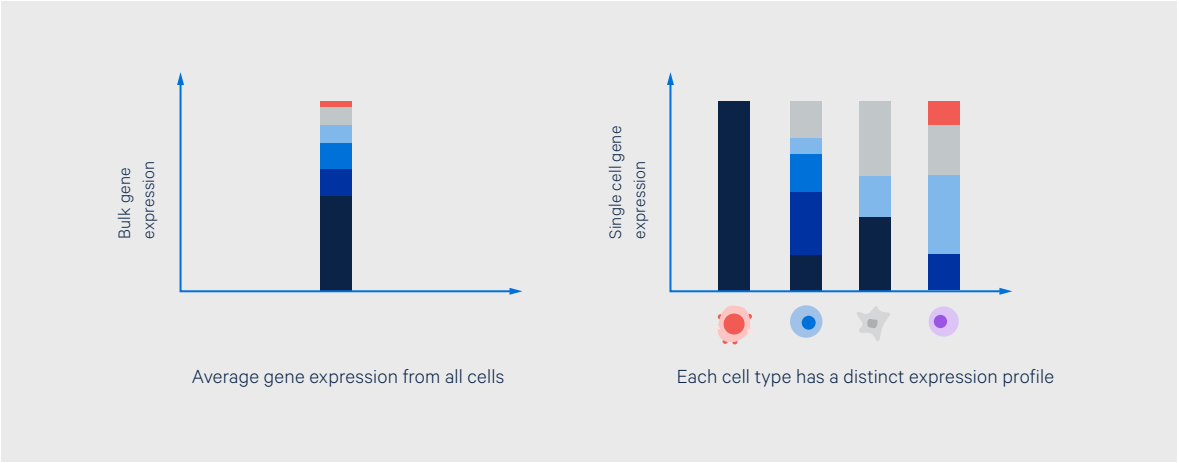


Figure 2. Increasing resolution, from bulk to single cell gene expression. The graph on the left displays a bulk view of the transcriptional landscape of a sample, with average expression levels for 6 genes. Representing single cell gene expression data, the graph on the right reveals the true cellular heterogeneity of the sample, as well as the cellular sources of the expression patterns seen from the average view.

Other techniques, including flow cytometry, can sort cells and provide insights into cellular identity through a single cell readout of a defined set of proteins. However, the expression patterns of select proteins may not be enough to capture the full picture of cellular heterogeneity or dynamic cell states, in much the same way that knowing someone's physical traits—height, hair color, eye color—can only provide a limited perspective of who they are.

Moving beyond descriptive analysis toward a functional understanding of the diverse cell types in a complex sample increasingly requires a *multiomic* perspective of cellular biology. Through single cell multiomics—the analysis and integration of datasets from different omic groups—scientists can measure multiple cellular features from the same single source. Such features include whole transcriptome gene expression, cell surface protein expression, immune repertoire sequences, including T- and B-cell receptors, and regions of open chromatin for a view of epigenomic regulation. With more information-rich data from a single experiment, researchers gain the additional advantages of preserving precious samples, increasing productivity, reducing errors caused by batch effects, and saving other high-commodity resources, from grant funds to personnel time.

Breakthroughs enabled by single cell multiomics

More than improving experimental processes, single cell sequencing methods are helping scientists make new inroads into the most crucial areas of health and disease research. Discoveries that would not have been possible with an averaged view of transcription or a single parameter readout are now changing how we understand and approach treatment for cancer, infectious disease, neurodegenerative conditions, and more.

Uveal melanoma (UM), a highly metastatic cancer largely unresponsive to checkpoint immunotherapy, had been extensively classified by bulk gene expression profiling, revealing tumor subtypes, their associated mutations, and correlation to patient outcomes. However, the genetic landscape of tumors could not reveal the specific factors that drove metastasis and resistance to immunotherapy. Hoping to develop a more complete understanding of UM progression, researchers from the

Comprehensive Cancer Center of the University of Miami turned to single cell RNA-sequencing, expanding the scope of their investigation to the tumor microenvironment (TME) as well. Single cell data revealed specific immune cell populations that had never been associated with UM before, as well as an alternative checkpoint marker, LAG3, that was more broadly expressed in UM tumors than the traditional CTLA4 and PD1 markers. These insights pointed to a previously unappreciated mechanism of cancer evolution, involving an interplay between tumor and immune populations in the TME that ultimately stratified tumor subtypes, as well as a hidden checkpoint marker driving therapeutic resistance (2).

"The genomic aberrations are in some sort of dance with the immune microenvironment and one is influencing the other and probably vice versa, meaning that the mutations and the chromosomal copy number variations are not acting in a vacuum; they're acting within this tumor microenvironment that altogether have to be understood and considered if we are to understand the process of metastasis in uveal melanoma."

- Dr. J. William Harbour, Vice Chairman for Translational Research, Director of Ocular Oncology, Bascom Palmer Eye Institute, Associate Director for Basic Research, Sylvester Comprehensive Cancer Center (3)

Using single-cell multiomics to reveal new insights into uveal melanoma evolution and therapeutic resistance

Explore the full study in this white paper from GenomeWeb and 10x Genomics.

Download now ⟶

High-impact discoveries across applications

Explore more discoveries enabled by single cell sequencing technology from 10x Genomics that are transforming how we understand biology and disease:

Novel cell types and states

"By associating cell type-specific expression programs with key disease genes, we establish a new cellular narrative for airways disease."
Montoro D, et al. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* 560: 319–324 (2018).

"scATAC-seq of tumor-infiltrating lymphocytes from patient biopsies identified regulatory programs controlling T cell exhaustion and a shared program with T follicular helper cells."
Satpathy A, et al. Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat Biotechnol* 37: 925–936 (2019).

"Meninges contain a pool of monocytes and neutrophils supplied not from the blood, but by adjacent skull and vertebral bone marrow. [...] These findings call for reinterpretation of immune-cell infiltration into the CNS during injury and autoimmunity and may inform future therapeutic approaches harnessing meningeal immune cells."
Cugurra A, et al. Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma. *Science* 373: eabf7844 (2021).

"We further reveal a specific cancer stem state that is significantly predictive of patient survival and can be used as a signature to identify high-risk patients..."
Guilhamon P, et al. Single-cell chromatin accessibility profiling of glioblastoma identifies an invasive cancer stem cell population associated with lower survival. *eLife* 10: e64090 (2021).

Disease mechanisms

"We observed not only increases in intratumoral heterogeneity, but the emergence of distinct cellular populations defined by established drug resistance gene signatures."
Stewart C, et al. Single-cell analyses reveal increased intratumoral heterogeneity after the onset of therapy resistance in small-cell lung cancer. *Nat Cancer* 1: 423–436 (2020).

"This study reveals, to our knowledge for the first time, that neuronal ApoE can act through neuronal MHC-I to elicit AD-related tau pathologies."
Zalocusky K, et al. Neuronal ApoE upregulates MHC-I expression to drive selective neurodegeneration in Alzheimer's disease. *Nat Neuro* 24: 786–798 (2021).

Therapeutic insights

"scRNA-seq analyses guided successful therapeutic intervention in the patient with refractory DiHS/DRESS. scRNA-seq may improve our understanding of complicated human disease pathophysiology and provide an alternative approach in personalized medicine."
Doyoung K, et al. Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: a case report. *Nat Med* 26: 236–243 (2020).

Research Snapshot

Targeted therapy guided by single cell transcriptomic analysis

Learn more ⟶

Therapeutic insights	
	"Unexpectedly, many antibody clones elicited by vaccination do not bind vaccine, demonstrating non-specific activation of bystander antibodies by influenza vaccination." Horns F, et al. Memory B Cell Activation, Broad Anti-influenza Antibodies, and Bystander Activation Revealed by Single-Cell Transcriptomics. *Cell Rep* 30: 905–913.e6 (2020).
	"Heterogeneity in the cellular and molecular features of CAR T cell infusion products contributes to variation in efficacy and toxicity after axi-cel therapy in large B cell lymphoma … day 7 molecular response might serve as an early predictor of CAR T cell efficacy." Deng Q, et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. *Nat Med* 26: 1878–1887 (2020).
	"The expansion of T cell clones did not derive from pre-existing tumor-infiltrating T lymphocytes; instead, the expanded clones consisted of novel clonotypes that had not previously been observed in the same tumor." Yost K, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat Med* 25: 1251–1259 (2019).
Drug discovery	
	**"Our results characterize transcriptional differences among SARS-CoV-2-specific B cells and uncover cross-neutralizing Ab targets that will inform immunogen and therapeutic design against coronaviruses."** Scheid J, et al. B cell genomics behind cross-neutralization of SARS-CoV-2 variants and SARS-CoV. *Cell* 184: 3205–3221.e24 (2021).

Table 1. Making the impossible possible. Statements pulled from recent publications leveraging single cell sequencing technology from 10x Genomics reveal the potential of a high-resolution view of biological complexity.

From identifying novel cell types and states, to unlocking the underlying mechanisms of disease and therapeutic response and resistance, single cell sequencing is fueling groundbreaking research with the potential to transform biology and medicine. Whether it's unexplained diseases or unexplored systems in the human body or nature, what was once blurry is now becoming clear. And much like Galileo's telescope, the tools at hand are allowing for increased focus and magnification as we move towards a future of science defined by new depths of insights that, only a decade ago, many could not have imagined.

References

1. Library of Congress, Digital Collections. Finding Our Place in the Cosmos: From Galileo to Sagan and Beyond. Galileo and the Telescope. https://www.loc.gov/collections/finding-our-place-in-the-cosmos-with-carl-sagan/articles-and-essays/modeling-the-cosmos/galileo-and-the-telescope
2. Durante M, et al. Single-cell analysis reveals new evolutionary complexity in uveal melanoma. *Nat Commun* 11: 496 (2020).
3. GenomeWeb and 10x Genomics. White Paper: Using Single-Cell Multiomics to Reveal New Insights Into Uveal Melanoma Evolution and Therapeutic Resistance.

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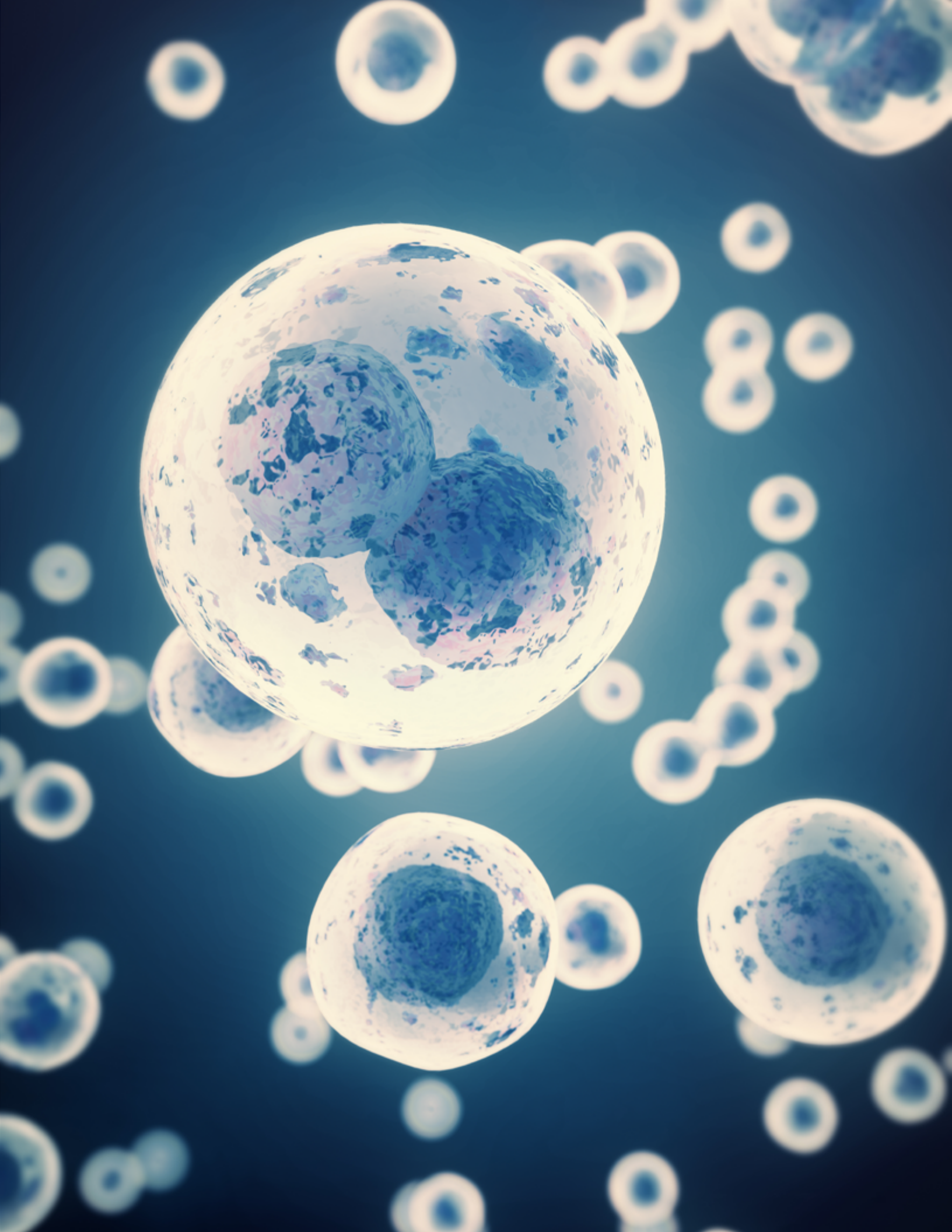

Chapter 2

Understanding a single cell sequencing experiment

Familiar steps with enhanced insights

As with learning any new technique, there may be elements of the single cell sequencing workflow that are currently unfamiliar to you. Though methods like flow cytometry, RNA-seq, and qPCR have already been established in your processes and institutions, the possibility of taking your research to the next level with a new method can offset the learning curve.

The differences between single cell sequencing and these methods—including the implementation of next-generation sequencing and data analysis—shouldn't be minimized, however there are a number of similarities between them that can give you confidence in your ability to step into the world of single cell sequencing. Past training and experiments, like muscle memory, contribute technical knowledge, hands-on skills, and intuition. These, combined with practice, will enable your success with a new method.

Just another experiment: Starting with sample prep

Every biological experiment, including single cell techniques, can be broken down into a few key steps: preparing a sample, running an assay, and looking at results.

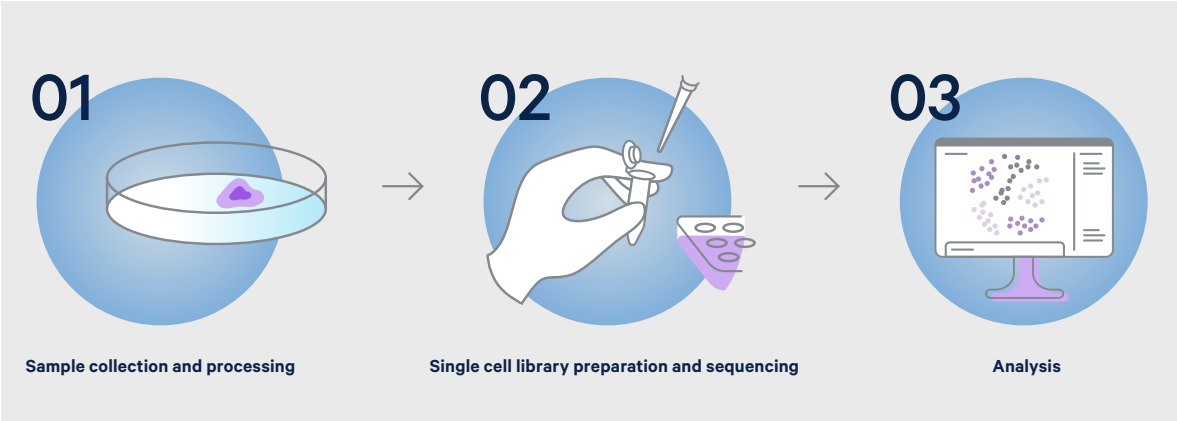

Figure 3. Three basic steps in every single cell experiment.

The first step in a single cell sequencing experiment, sample preparation, aligns closely with what you may already be familiar with as a flow cytometry user—namely, generating viable single cell suspensions from whole samples that have been digested through an enzymatic or mechanical process, cell sorting, or other cell isolation techniques. This is followed by cell counting and quality control steps to ensure your sample has an appropriate concentration of viable cells and is free of clumps and dead cell debris. If desired, you can also stain your sample with antibodies to label cell surface proteins and other biological analytes, or perform FACS enrichment for cell types of interest.

The steps taken to dissociate a sample will vary based on your starting material and the goals of your experiment. Additional preparation steps may be necessary depending on the quality of the tissue, sample abundance, cell size, or the need to extract nuclei for chromatin accessibility profiling from genomic DNA. Regardless of these specific considerations, the same basic principle of generating a high-quality single cell suspension applies across sample types.

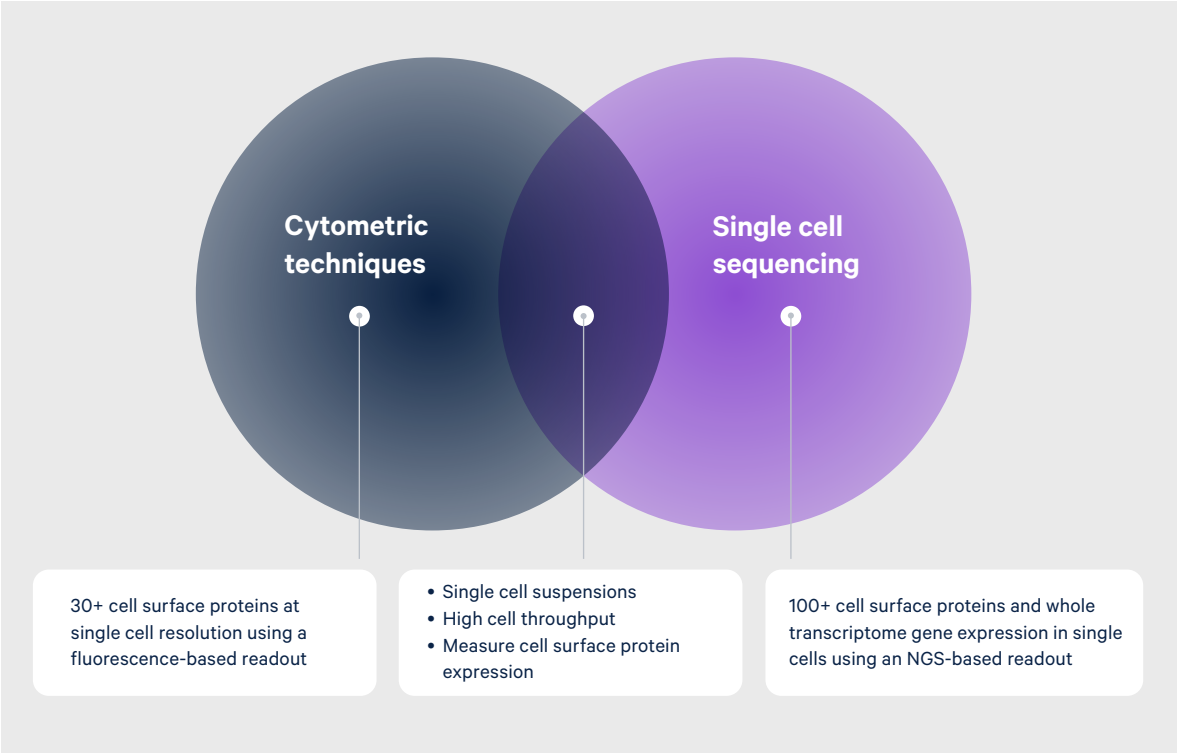

Figure 4. A depiction of the similarities and differences between cytometric techniques, such as flow cytometry, and single cell sequencing technology from 10x Genomics.

Gene expression three ways: It's all about the cDNA

Single cell RNA-seq, RNA-seq, and reverse transcription qPCR (RT-qPCR) share a common process of isolating RNA and converting it to cDNA for analysis. Each assay differs, however, in regards to how this process is performed and the final readout. Both RNA-seq and RT-qPCR start by isolating RNA from a population of cells in bulk and then converting it to cDNA. For RNA-seq, the cDNA is used to create a next-generation sequencing library, enabling measurement of gene expression across the entire transcriptome. For RT-qPCR, specific targets are amplified from the cDNA and expression levels measured by fluorescence. While RT-qPCR is limited to known targets, RNA-seq provides unbiased whole transcriptome gene expression. Both, however, only provide a view of the average gene expression across a population of cells. With single cell RNA-seq, single cells are isolated into wells or individual micro-reaction vessels before the RNA is isolated. The RNA is then labeled with cell-specific barcodes, ensuring cDNA from each cell can be traced back to the cell of origin. Similar to RNA-seq, the barcoded cDNA is used to create a next generation sequencing library, enabling measurement of gene expression across the entire transcriptome of each individual cell.

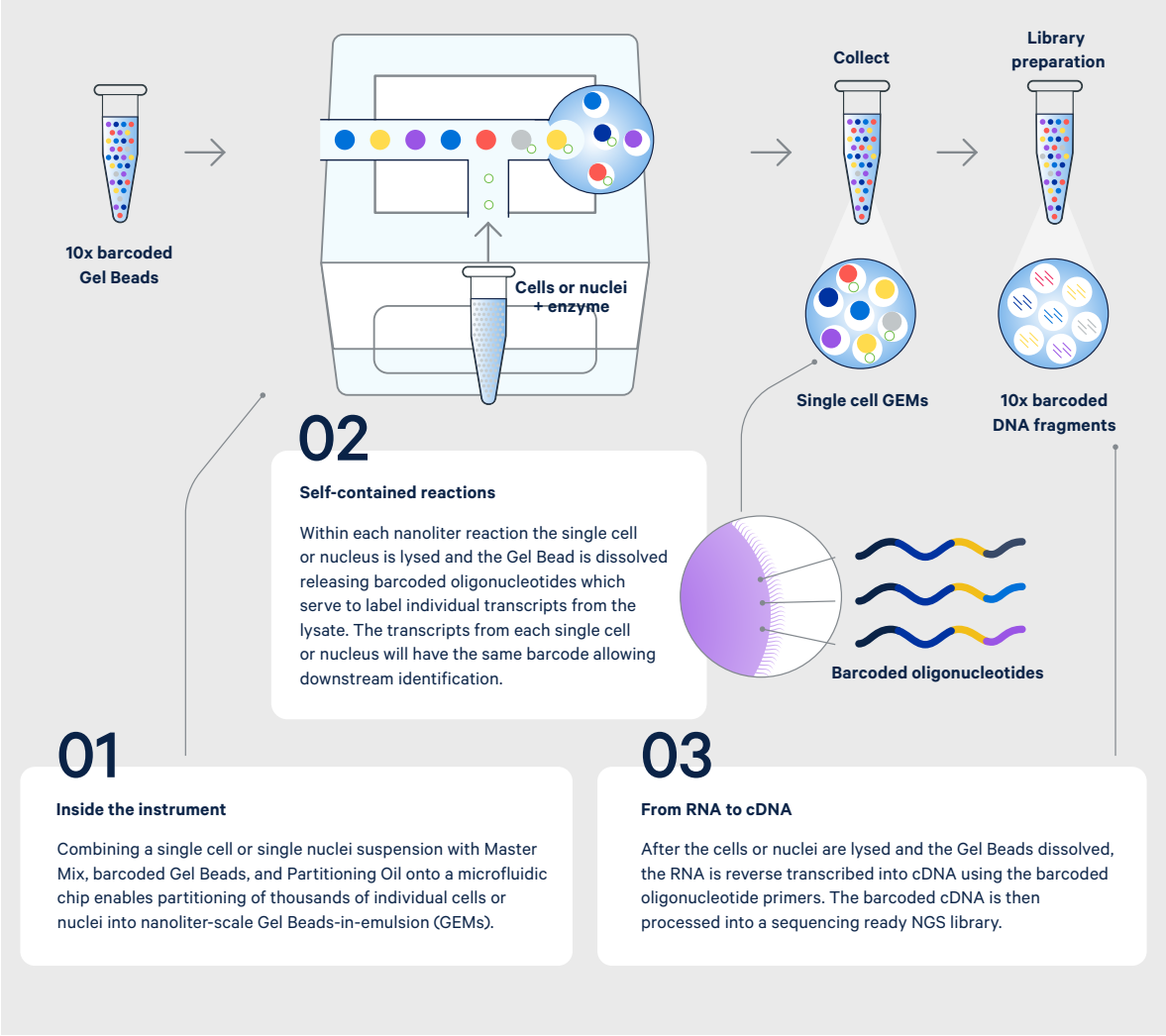

Figure 5. How it works: single cell gene expression, from sample to sequencing library.

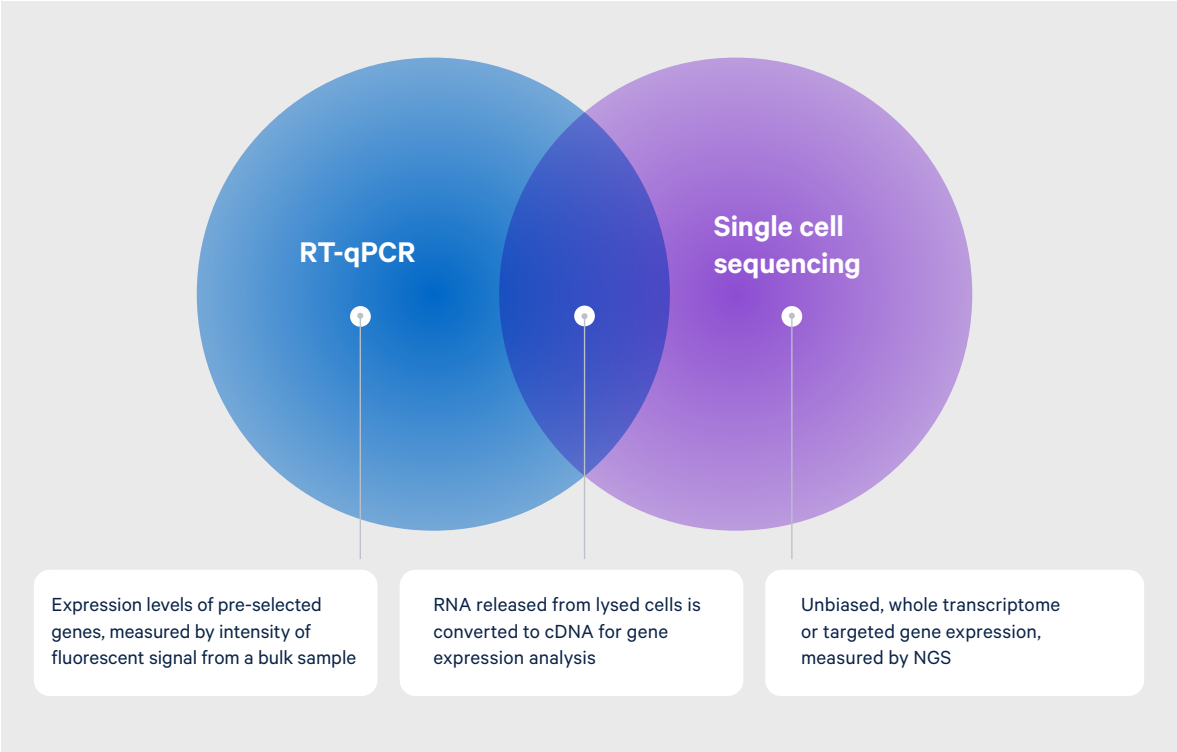

Figure 6. A depiction of the similarities and differences between RT-qPCR and single cell sequencing technology from 10x Genomics.

A common goal: Understanding the transcriptome

Whether starting with RNA from bulk samples for RNA-seq or RT-qPCR, or from single cells for single cell RNA-seq, each technique is ultimately looking at the same endpoint data: levels of gene expression.

Among these assays, there is a common approach to data analysis, including analyzing differential gene expression between experimental or sample conditions, such as normal versus diseased. For example, comparing the transcriptional profile of a control sample and inflamed skin tissue can reveal transcriptional signatures that define the inflammatory state and the gene activity that is dysregulated in the disease context. With single cell resolution, you can take these transcriptional insights to the next level, identifying whether there is a specific subset of cells responsible for the inflammatory response.

Leveraging known gene expression markers from your experiences of quantifying gene expression through RNA-seq and RT-qPCR experiments, and a familiar mode of differential gene expression analysis—similar to that of both RNA-seq and RT-qPCR data—the jump to single cell gene expression can be very intuitive. Additionally, 10x Genomics software can help you make a smooth transition to single cell data, with easy-to-use tools for data analysis and visualization.

Have more questions about how to analyze single cell sequencing data? Jump to Chapter 5 for an introduction to our tools and how to find support.

Jump forward ⟶

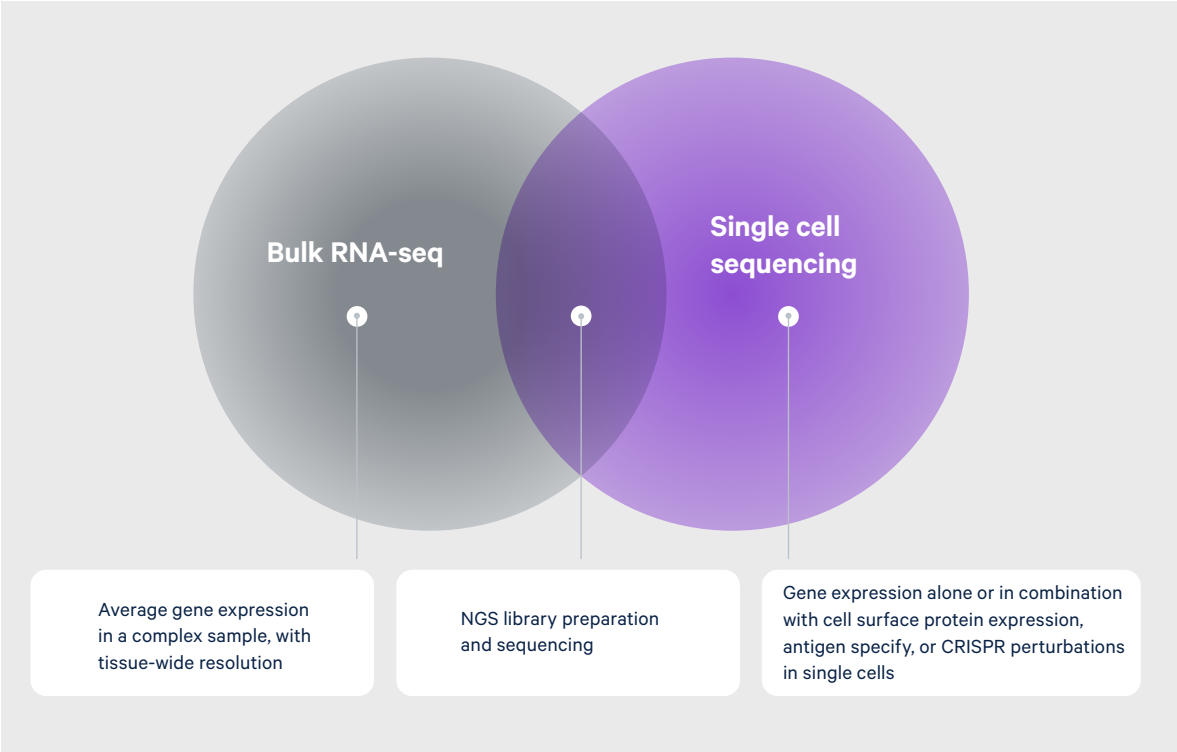

Figure 7. A depiction of the similarities and differences between bulk RNA-seq and single cell sequencing technology from 10x Genomics.

A primer on primers: How next-generation sequencing works for single cell experiments

We'd like to provide a short overview of how next-generation sequencing libraries are constructed. First, next-generation sequencing (NGS) is a high-throughput method to determine DNA and RNA sequences, with specific application to finding variation in sequences and defining gene expression patterns. Single cell RNA sequencing sequencing allows you measure gene expression levels by sequencing and counting transcripts. NGS libraries are prepared by adding sequencing specific adaptors to the ends of cDNA fragments via PCR. This creates a library of cDNA fragments labeled with both 10x-specific sequences for mapping transcripts back to their cell of origin and sequencing primers used for the reaction within the sequencing instrument.

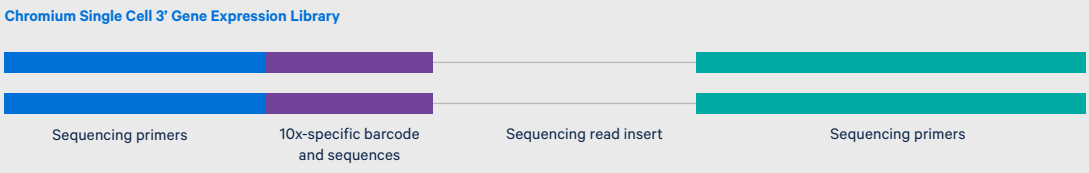

Figure 8. A simplified diagram of a DNA fragment with sequencing primers attached on both ends.

Elevating insights with single cell multiomics

A sequencing-based readout is ultimately powerful because it can provide access to multiple biological analytes from the same single cell. If single cell gene expression provides highly detailed information in one color, then single cell multiomics offers the same detail but across the full color spectrum, giving you the most true-to-life view of your sample.

Continued innovation is allowing the 10x Genomics Chromium Single Cell platform to provide a readout of multiomic features of cellular complexity from the same single cells, including many of the analytes described in the figure to the right. As opposed to traditional methods, single cell multiomics streamline the experiments you need to run and the samples you need to analyze to get equivalent results. This not only conserves precious samples, but also ensures greater biological accuracy, as you do not need to match datasets from split samples and infer relationships between omic types computationally.

The end result of a single cell sequencing experiment is well worth the effort to understand and become competent in running it. With the experiment done and data in hand, discovery begins.

References

1. Wagner E. Monitoring gene expression: quantitative real-time rt-PCR. *Methods Mol Biol* 1027: 19–45 (2013).
2. https://www.illumina.com/science/technology/next-generation-sequencing.html
3. https://www.illumina.com/science/technology/next-generation-sequencing/beginners/ngs-workflow.html

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Gene expression

Perform molecular and cellular characterization of cells at scale with single cell RNA-sequencing for whole transcriptome or targeted gene expression

Immune profiling

Gain a comprehensive view of the immune system with paired, full-length receptor sequences from T and/or B cells, surface protein expression, antigen specificity, and gene expression, all from a single cell.

Epigenomic profiling

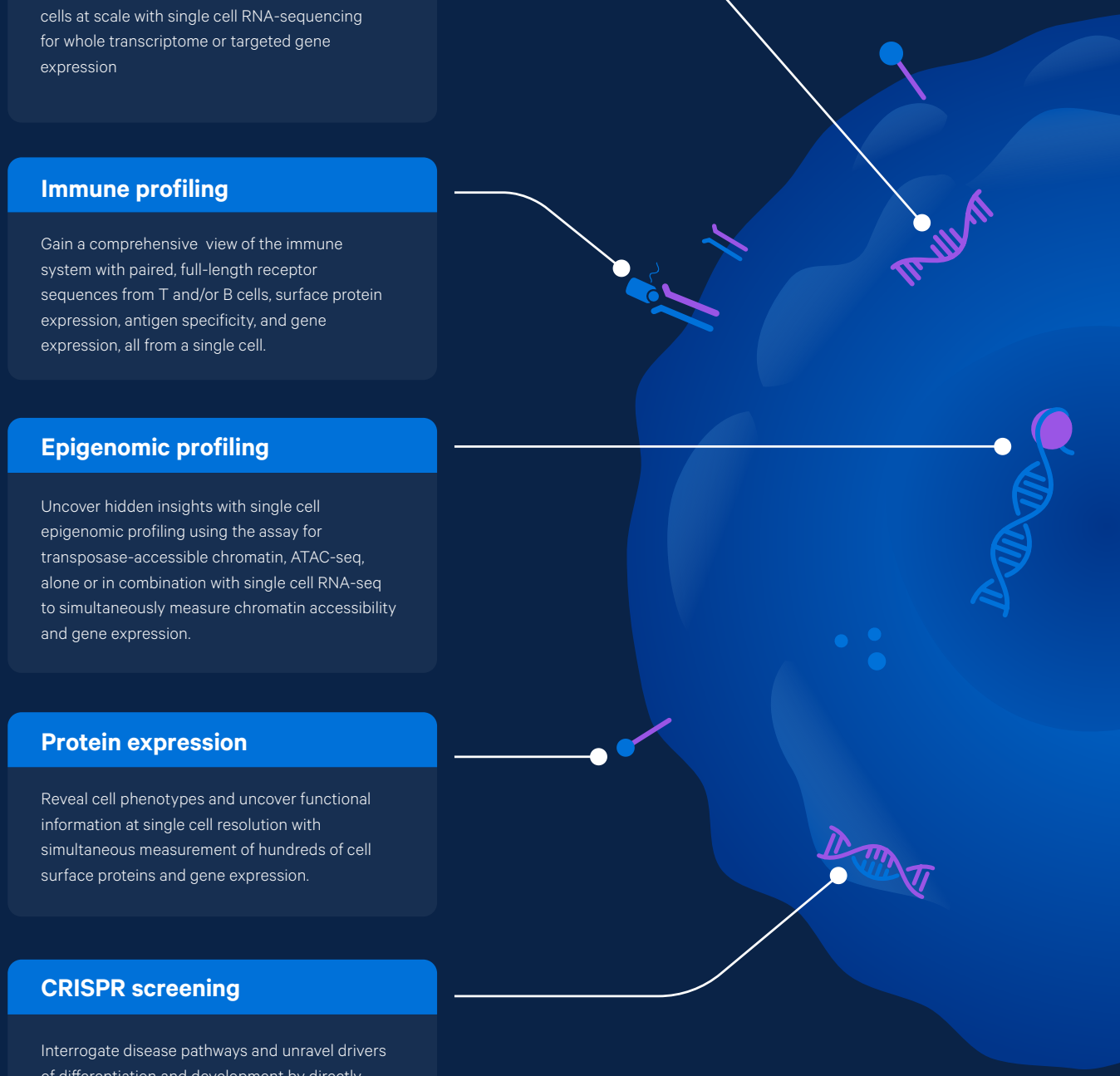
Uncover hidden insights with single cell epigenomic profiling using the assay for transposase-accessible chromatin, ATAC-seq, alone or in combination with single cell RNA-seq to simultaneously measure chromatin accessibility and gene expression.

Protein expression

Reveal cell phenotypes and uncover functional information at single cell resolution with simultaneous measurement of hundreds of cell surface proteins and gene expression.

CRISPR screening

Interrogate disease pathways and unravel drivers of differentiation and development by directly linking CRISPR edits and gene expression phenotypes at single cell resolution, across hundreds of genes in tens of thousands of cells.



= mRNA

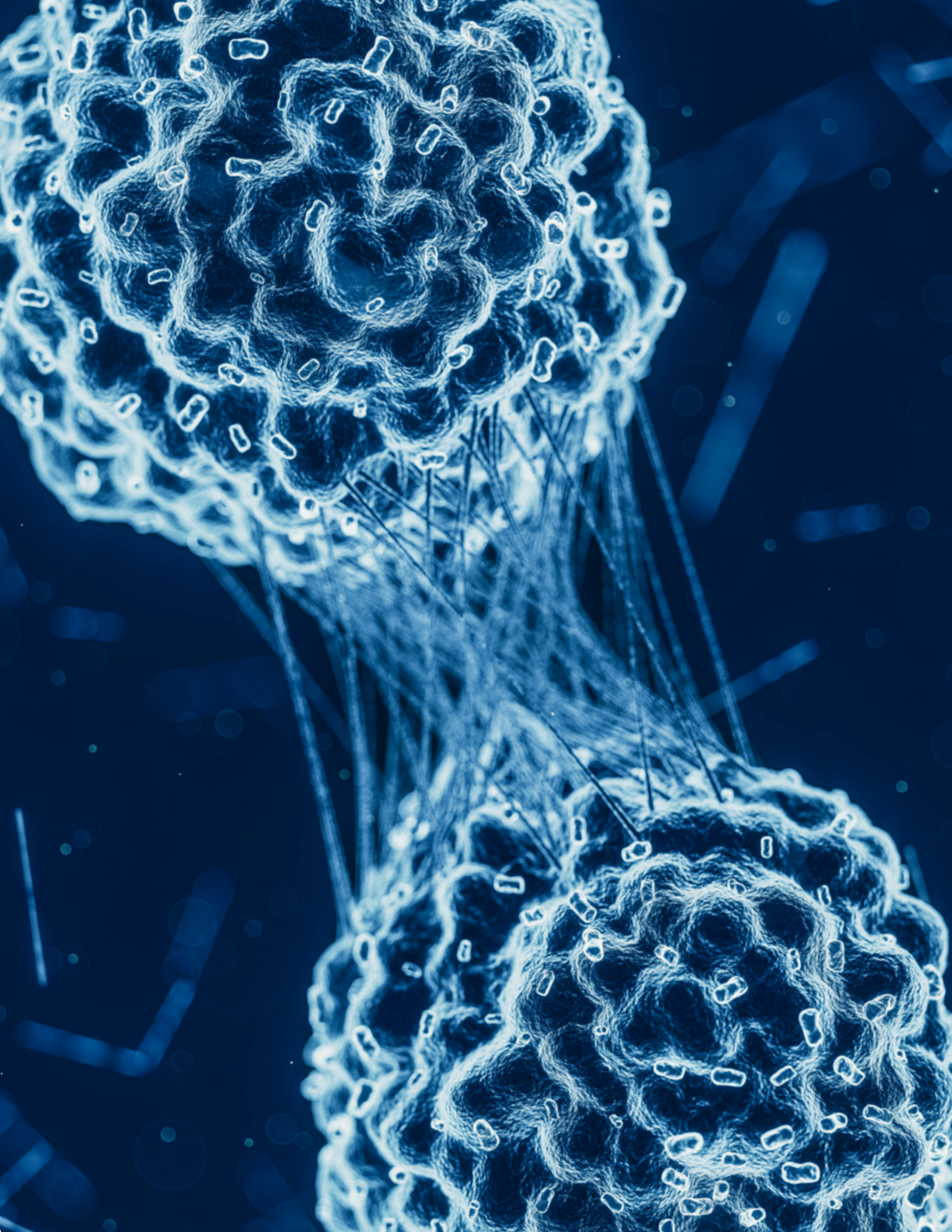
= CRISPR perturbation

= Chromatin accessibility

= T-cell receptor

= Cell surface protein

Figure 9. Access multiomic analytes from the same single cell.



Chapter 3

Comparing single cell sequencing methods

Considerations for your goals

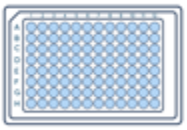
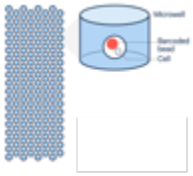
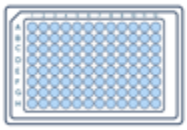
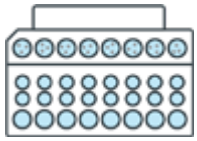
They say variety is the spice of life, but having to dig through all the academic and commercially available single cell sequencing methods you could use may seem daunting. In this chapter, we'll help you sort through a few options. It will be important to understand what each respective method offers in terms of throughput, depth of insights, instrumentation requirements, and hands-on experimentation. Knowing this can help you determine the single cell sequencing platform that best suits your research goals.

Different approaches to single cell sequencing

The lineup of currently available single cell sequencing technologies differ from one another in a few main areas. The first is the physical platform used to partition single cells into contained micro-reactions. This is the space where individual cellular contents are released and transcripts or other biological analytes are tagged for downstream identification, or indexed. How this indexing occurs is another major difference between available technologies, as are the workflow steps involved in generating a sequencing-ready library.

There are far too many approaches to cover here so we'd like to focus on summarizing the four most popular single cell sequencing platforms used by researchers today.

At-a-glance comparison

Technology	Plate-based	Nanowell	Combinatorial barcoding	Droplet-based (10x Genomics)
Overview	FACS-sorted single cells are separated into individual wells of reaction plates where the cells are lysed and undergo strand synthesis and tagmentation to construct cDNA and sequencing libraries.	Barcoded mRNA capture beads and single cells are distributed in an array of sub-nanoliter wells. Single cells are isolated into individual nanowells by gravity.	Pools of cells split into multi-well plates and transcripts are indexed with well-specific barcodes. Process is repeated 3–4 times to generate a unique combinatorial barcode for each cell.	Single cells are encapsulated in individual partitions, each containing all the necessary reagents for cell lysis, reverse transcription, and molecular tagging.
Platform	96-well reaction plates 	Nanowell array 	96-well reaction plates 	Microfluidics chip 
Additional required equipment	FACS instrument Separate reagents	Nanowell array chips Kitted or individual reagents	No customized equipment or instrument Kiited or individual reagents	Chromium instrument (including Chromium Controller, Connect, or X/iX) Kitted solution contains all enabling reagents
Throughput	Low throughput 384 cells/run 1 sample per run	Moderate throughput 100 to 40,000 cells/run 1 sample per run without sample multiplexing 12 samples with multiplexing	Low to high throughput 100,000 cells/run 1-48 samples for kitted solutions	Low to high throughput 100 to 320,000 cells/run without sample multiplexing 1–16 samples without sample multiplexing 100 to 730,000 singlets* per run with sample multiplexing 1–192 samples per run with sample multiplexing *Singlets are single cells or nuclei captured after multiplet removal
Cell recovery	N/A	Up to 70%	30–50%	Up to 65%
Cell size limit	N/A	≤ ~20 µm	N/A	≤ ~50 µm
Reaction volume	N/A	Nanoliter	N/A	Nanoliter
Transcript coverage	Full length	3’	3’	3’ or 5’
Immune receptor profiling	Yes	Yes (platform dependent)	No	Yes

Technology	Plate-based	Nanowell	Combinatorial barcoding	Droplet-based
Multiomic analysis	No	Yes	No	Yes
Unbiased, whole transcriptome analysis (WTA) vs. targeted gene expression	WTA	Targeted and WTA (platform dependent)	WTA	Targeted and WTA
Length of workflow	~2 days	~1.5 days	~2 days	~1 day

Understanding the process

Each of these powerful sequencing methods offer their own advantages. However, choosing the method that's right for you also comes down to qualities that can't always be reflected on paper. What is it like, experientially, to run the experiment? How many steps are involved? How complicated are those steps, or are they prone to introduction of error? Am I supported at potential stalling points in the experiment or in data analysis?

As you continue to weigh the various costs and benefits of each method, it will be important to factor in what is feasible for your sample, your own time or personnel resources, your institution, and more. What technology can best suit your biological questions and the scope of your experimental ambitions? These considerations will help to ensure you can make an informed decision that you're happy with in the end.

Questions for consideration

1. What scientific questions do I want to answer?
2. What can I do with my available sample?
    a. Sample type and processing
    b. Number of cells
    c. Cells versus nuclei
3. How many cells and replicates do my experiments require?
    a. Cell throughput
    b. Scale
4. What are my resourcing or price constraints?
5. What experiment will fit best into my current workflows and processes?

If you'd like to continue exploring the differences between these sequencing methods, including relative performance, the following review papers are great starting points:

- Ashton J, et al. Comparative analysis of single-cell RNA sequencing platforms and methods. *bioRxiv* (2020). Read more
- Ding J, et al. Systematic comparison of single-cell and single-nucleus RNA-sequencing methods. *Nat Biotechnol* 38: 737–746 (2020). Read more
- Haque A, et al. A practical guide to single-cell RNA sequencing for biomedical research and clinical applications. *Genome Medicine* 9: 75 (2017). Read more
- Mereu E, et al. Benchmarking single-cell RNA-sequencing protocols for cell atlas projects. *Nat Biotechnol* 38: 747–755 (2020). Read more

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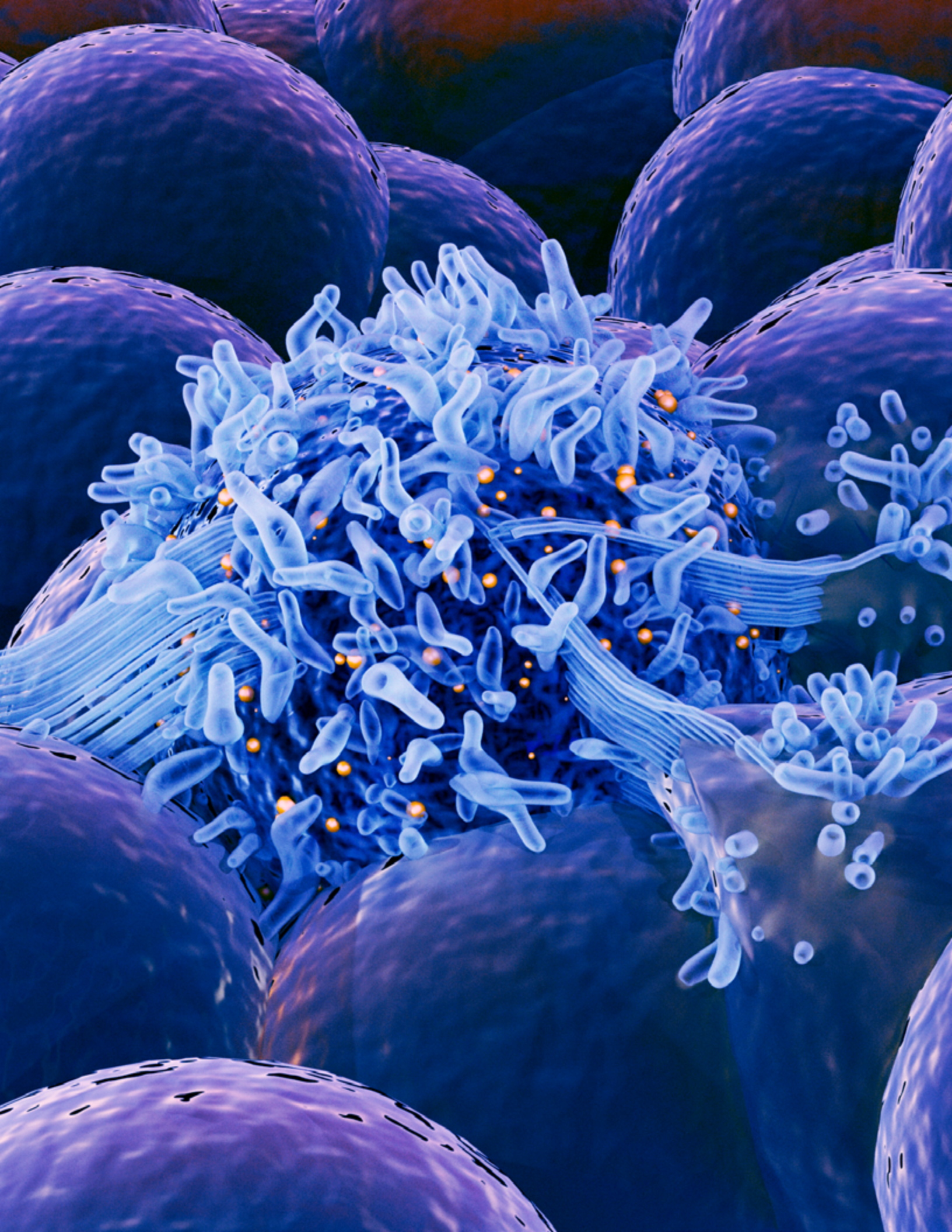

Chapter 4

Single cell discoveries across the life sciences

Resolving biology to advance human health

"When I think of innovation, you could immediately think of a brand new technology that's a game changer—that's paradigm shifting. But I also think, within science, it's about advancement. Every scientist is an innovator in a way, because if you're advancing knowledge, that's innovation."

- Dr. Samantha Bucktrout, Senior Director of Research, Parker Institute for Cancer Immunotherapy (1)

Scientific research is at its best when collaboration and shared technological innovations feed off each other to drive new discoveries. One project peels back a layer of complexity; the next project peels back another layer, together building a more complete understanding of biology.

As you consider taking advantage of single cell tools from 10x Genomics for your research, keep in mind that you're not alone. You are part of an ecosystem of researchers who have already begun to peel back the layers of complexity unique to your area of study, and who have demonstrated the utility of single cell sequencing methods to address biological questions that matter to your field. With over 2,400 peer-reviewed publications in major journals across the fields of oncology, neuroscience, immunology, developmental biology, and more, 10x Genomics single cell technology has been established as a critical assay for enabling leading-edge life science research.

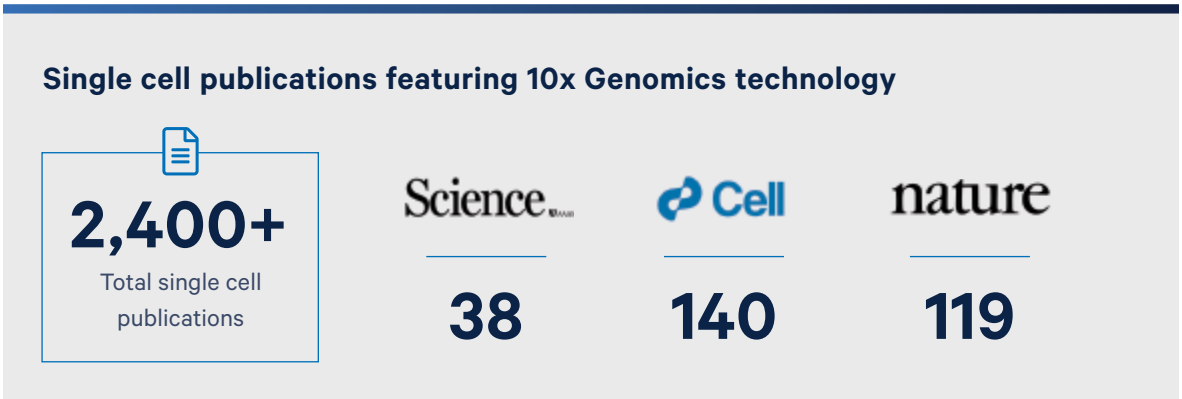

Figure 10. An increasing number of scientists are using 10x Genomics tools to publish high-impact research in top journals.

Explore the following sections to find applications of single cell technology to your field of interest, and review published research that can serve as inspiration for the kinds of experiments you may also want to do.

Resolving cancer with single cell multiomics

Traditional approaches have enabled researchers to make great strides in understanding the complexities of cancer biology, and to translate groundbreaking discoveries into lifesaving cancer treatments and therapies. However, many unanswered questions remain, requiring increased scale and resolution to be addressed. What mechanisms underlie differences in tumor development, progression, and metastasis in different individuals? How can varying responses to therapies be predicted across cancer types and patients?

"The promise of therapeutically targeting self-renewing tumor-initiating cancer cells depends on our capacity to capture the full range of heterogeneity within this population from individual tumors."

- Guilhamon P, et al. Single-cell chromatin accessibility profiling of glioblastoma identifies an invasive cancer stem cell population associated with lower survival. eLife 10: e64090 (2021). (2)

Single cell multiomics can illuminate the details and dynamics of this complicated disease, providing a holistic view of the immune and tumor microenvironment contexture, unmasking tumor heterogeneity, and pinpointing new cellular and molecular signatures of therapeutic response and resistance.

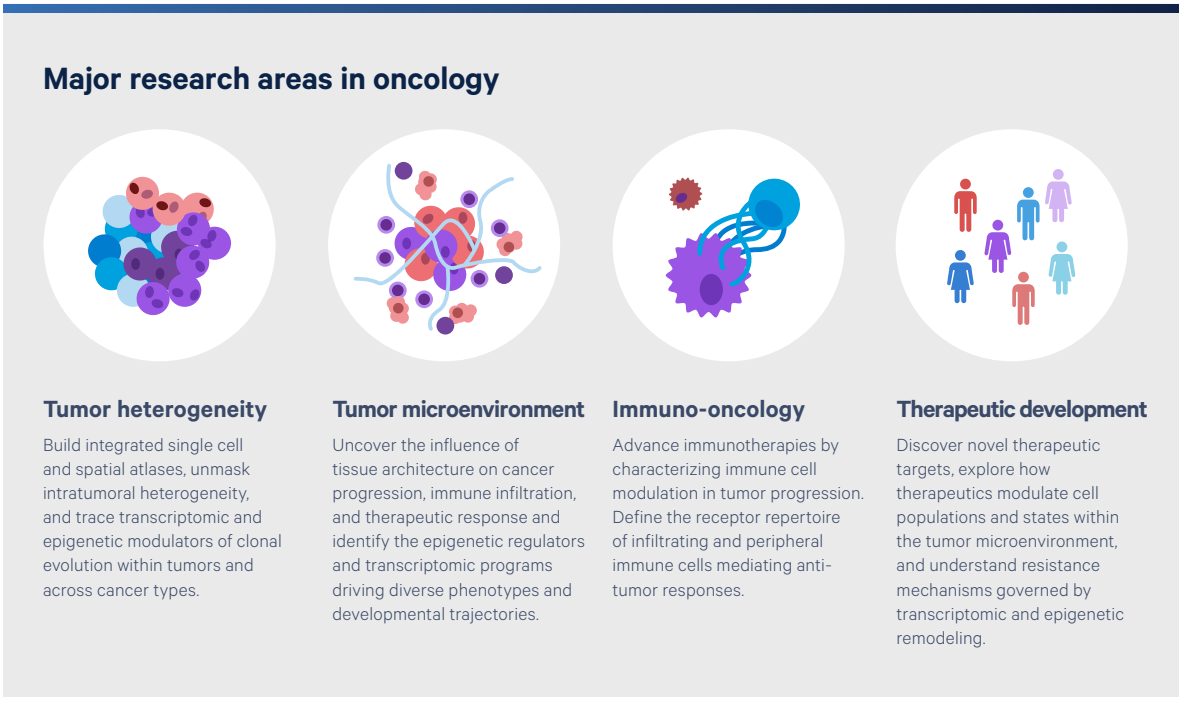

Figure 11. Applications of single cell multiomics for oncology research.

Oncology resources hub

Exponential hope

"Everytime we get a new dataset we're finding brand new things that we had no idea existed before—new types of T cells, new ways that cancers are learning to get around the immune system."

Fred Hutchinson Cancer Research Center
Seattle, WA

Paulson KG, et al. Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. Nat Commun 9(1): 3868, 2018.

Explore the study ⟶

Resolve cancer with single cell and spatial multiomics

Download our brochure to explore applications of 10x Genomics technology to oncology research, from defining the heterogeneity of the tumor immune microenvironment to advancing cancer therapy.

Download now ⟶

Explore more single cell applications for oncology research at 10xgenomics.com/cancer.

Featured publications

- Bi K, et al. Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma. *Cancer Cell* 39: 649–661.e5 (2021). Read more
- Satpathy A, et al. Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat Biotechnol*, 37: 925–936 (2019). Read more
- Zavidij O, et al. Single-cell RNA sequencing reveals compromised immune microenvironment in precursor stages of multiple myeloma. *Nat Cancer*, 1: 493–506 (2020). Read more

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Making your mark on the future of immunology

Immunology research is at the forefront of many important scientific and medical breakthroughs, including profiling emerging pathogens such as SARS-CoV-2, developing vaccines, development of immuno-oncology therapies, understanding autoimmune diseases, tissue matching for safe organ transplantation, and beyond. Today, immunology and infectious disease researchers continue to advance our understanding of these crucial applications to human health. However, their efforts face challenges due to the complex and dynamic nature of the immune system and the limitations of prevailing research tools that typically rely on cell surface markers to characterize immune heterogeneity.

The biggest challenges require the most innovative solutions. In order to comprehensively monitor and understand an immune response, scientists need the ability to characterize cell types and functional states in individual cells at both multiomic resolution and high throughput. Advancements in single cell sequencing are paving the way for the next generation of breakthroughs, allowing researchers to make their mark on the future of immunology.

"Having the ability to look at thousands of single cells and hundreds or thousands of genes is really revolutionizing immunology. It's allowing us to ask questions and discover therapies faster than we would have ever thought."

- Dr. Steven Bosinger, Assistant Professor, Emory University, Director of Genomics Laboratory, Yerkes National Primate Research Center (1)

Immunology resources hub

Breaking new ground in immunology: Through the lens of single cell and spatial multiomics

Dive into innovative research across key immunology research areas in this eBook.

Download now ⟶

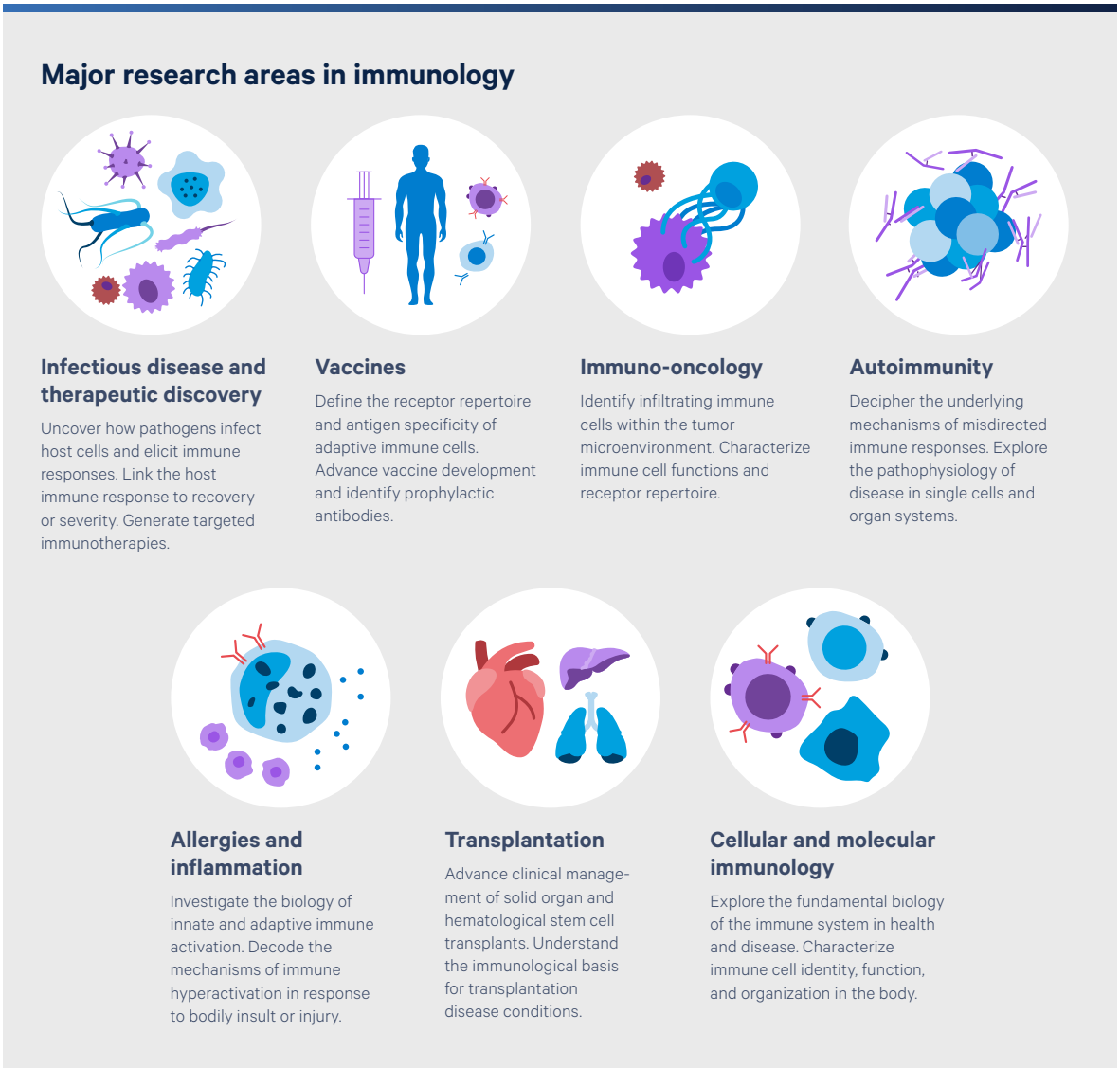

Figure 12. Applications of single cell multiomics for immunology research.

Featured publications

- Wimmers F, et al. The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination. *Cell* 184: P3915–3935. E21 (2021). Read more
- Dugan HL, et al. Profiling B cell immunodominance after SARS-CoV-2 infection reveals antibody evolution to non-neutralizing viral targets. *Immunity* 54: 1290–1303.e7 (2021). Read more
- Zhang W, et al. A framework for highly multiplexed dextramer mapping and prediction of T cell receptor sequences to antigen specificity. *Sci Adv* 7: eabf5835 (2021). Read more
- Park JE, et al. A cell atlas of human thymic development defines T cell repertoire formation. *Science* 367: eaay3224 (2020). Read more

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Reimagining neuroscience with single cell multiomics

The nervous system is a complex network of diverse cell types with a myriad of functions, communicating via dynamic signaling pathways and synaptic interactions. Neural cells, comprising excitatory and inhibitory neurons, and glial cells, including astrocytes, oligodendrocytes, ependymal, and microglial cells, interact with each other and with cells outside of the central nervous system in a delicate balancing act.

When any part of these intricate functions are disrupted—through trauma, disease, or genetic variation—neurological disease and disorders can result. Ongoing research is aimed at understanding the causative mechanisms of these disorders, with the ultimate goal of developing improved treatment options. This relies on the ability to carefully identify cell types within the nervous system and understand molecular changes that drive disease progression and treatment response. Single cell multiomic technology empowers neuroscientists to reveal the full complexity of neural diversity, and so gain a richer understanding of normal brain function and neurological disease states.

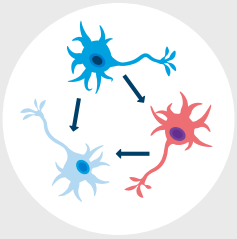
"I look at how astrocytes actually respond to inflammatory insults. And, you know, the only reason we can do this is because we can now zoom in at single cell resolution and look at how inflammation affects different subtypes of astrocytes. That's really key because it turns out that the actual changes that occur in astrocytes are restricted or particularly strong in a very, very small subset."

- Dr. Philip Hasel, Postdoctoral Fellow, Neuroscience Institute, NYU Grossman School of Medicine (3)

Major research areas in neuroscience

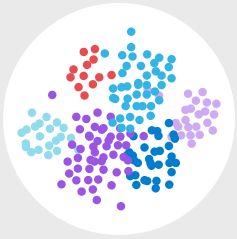
Neurodegenerative disease

Build a complete picture of the complex biology of neurodegenerative diseases in multiple dimensions. Advance your understanding of how pathological features affect the surrounding microenvironment and deepen your insights into the cellular contribution to disease pathogenesis and progression.



Psychiatric disorders

Discover the molecular foundations of psychiatric disorders with single cell and spatial technologies. Characterize disease-associated cellular phenotypes and uncover novel pathological effects. Explore the regulatory landscape of neural cells and identify epigenetic regulators of discrete cell types and states.



Developmental neuroscience

Achieve a more detailed view of neural development and differentiation than previously possible. Dissect molecular pathways controlling cellular differentiation, identify novel cell subtype markers, and reveal the dynamic gene expression patterns and regulatory landscapes governing cell differentiation.

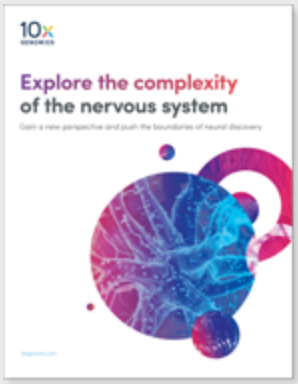
Figure 13. Applications of single cell multiomics for neuroscience research.

Neuroscience resources hub

Explore the complexity of the nervous system: Gain a new perspective and push the boundaries of neural discovery

Read the story of single cell and neuroscience research in this eBook, including the discoveries that are now possible with multiomic technologies.

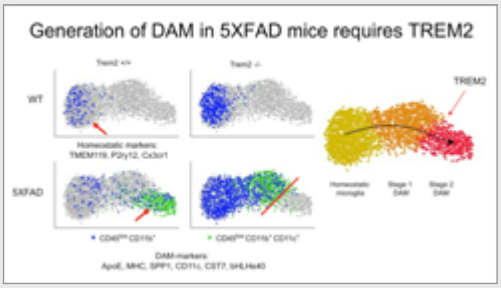
Download now ⟶



Harnessing TREM2 in the therapy of neurodegeneration and cancer

Learn about the role of Trem2 in the microglia response to Alzheimer's disease pathology, and new ways scientists are leveraging Trem2 for cancer therapy.

Watch now ⟶



Explore more single cell applications for neuroscience at 10xgenomics.com/neuroscience.

Featured publications

- Brioschi S, et al. Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders. *Science* 373: eabf9277 (2021). Read more
- Da Mesquita S, et al. Meningeal lymphatics affect microglia responses and anti-Aβ immunotherapy. *Nature* 593: 255–260 (2021). Read more
- Dani N, et al. A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* 184: 3056–3074.e21 (2021). Read more

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1. 10x Genomics. Science of Tomorrow. https://www.10xgenomics.com/science-of-tomorrow/.
2. Guilhamon P, et al. Single-cell chromatin accessibility profiling of glioblastoma identifies an invasive cancer stem cell population associated with lower survival. *eLife* 10: e64090 (2021).
3. 10x Genomics. Science of Tomorrow. https://www.10xgenomics.com/science-of-tomorrow/. Neuroscience Q&A with Dr. Philip Hasel and Dr. Shane Liddelow, Neuroscience Institute, NYU Grossman School of Medicine.

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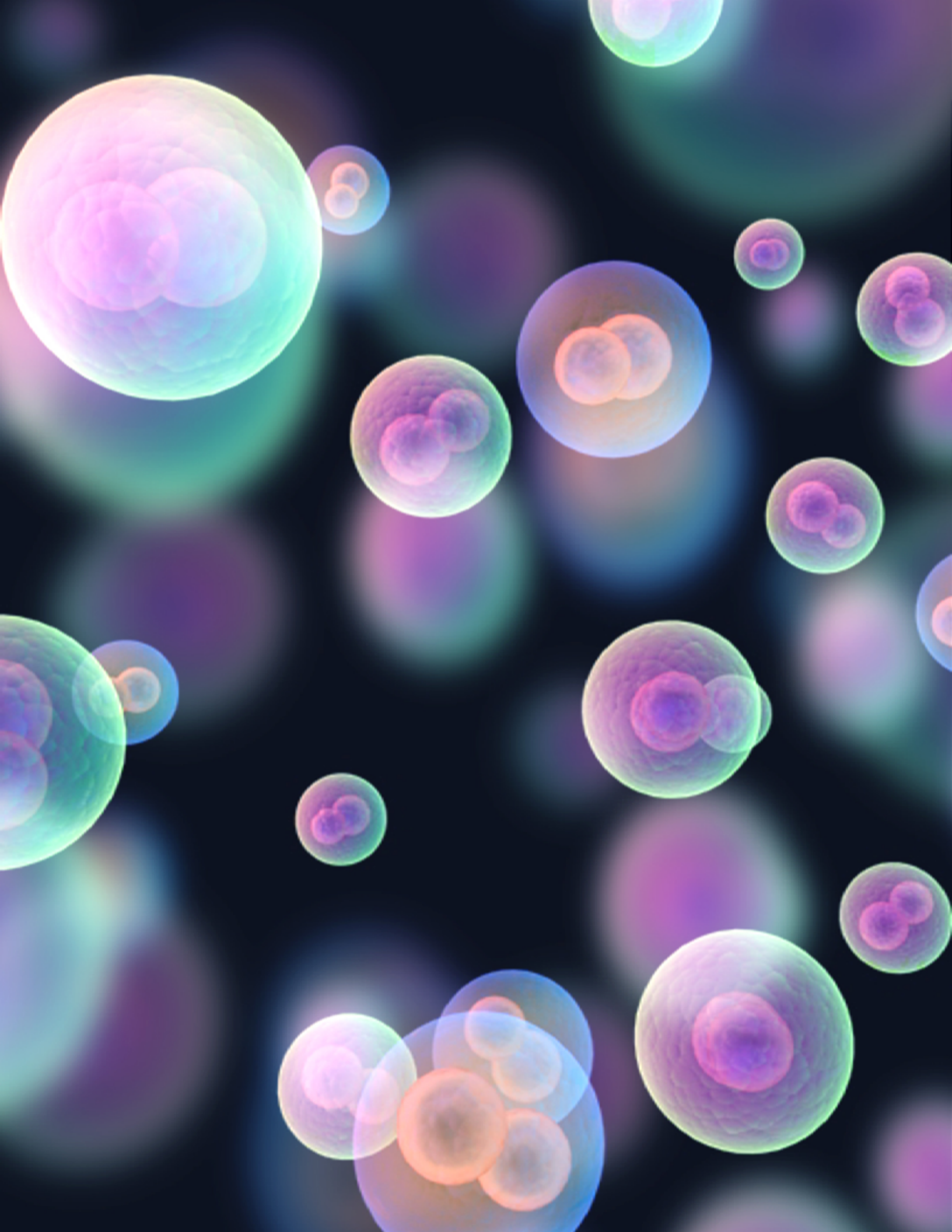


Chapter 5

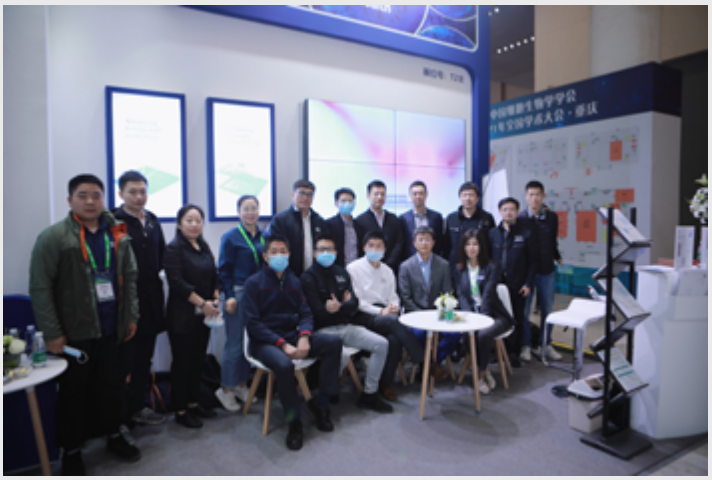
Supporting the start of your single cell journey

Addressing frequently asked questions

Embarking on your first single cell sequencing experiment is a process. It starts with becoming familiar with the technology, how it works, and the kinds of insights it can provide that match your research questions. Experimental planning will be a very important part of that overall process as well. A journey that begins with a clear trajectory—where are you going and how are you going to get there—will be more likely to reach its end without mishaps. This includes coordinating sample collection, understanding the necessary steps of your sample prep and experimental protocol, and the relative timing of these steps. Ensuring competency in running the assay and anticipating the basic knowledge required for performing data analysis will also be important for success.

We have confidence in your adaptability and skills to take on this new experiment type. And you won't be alone. 10x Genomics provides world-class technical and software support that is available by email or phone when you need it. Additionally, our knowledgeable field team often coordinates steps across the research journey, whether providing guidance for experimental design or connecting scientists with one another for collaborative studies. With that support, we want to assure you of our constant presence as your scientific partner through the experimental process.

Novel cell types implicated in COVID-19 cytokine storm: Findings from the Single Cell Consortium for COVID-19 in China



Researchers from 39 institutes and hospitals across China came together to deeply profile the immune response to SARS-CoV-2 infection, collectively performing single cell RNA-sequencing on 284 samples from 196 COVID-19 patients and controls, totaling 1.46 million cells.

Find out how 10x Genomics scientists played a part in enabling the success of this team effort, from connecting labs in the midst of a global pandemic to providing guidance and reagents.

Read more on the 10x Genomics Blog ⟶

Now, more than ever, making a personal connection with someone who can walk you through your questions will be incredibly beneficial. We'd encourage you to use our Contact Us page to engage a technical specialist in your region who can be that voice of support and guidance as you begin to plan your experiments.

There are a few frequently asked questions that we thought might also be helpful to answer here for you.

Frequently asked questions

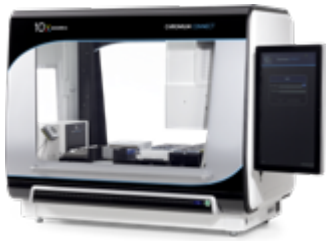
1. How do I know which 10x Genomics single cell product is right for me?

This is something that you can talk to your sales representative about, as they will have comprehensive knowledge of the 10x Genomics portfolio. Additionally, we recommend reviewing our product offerings with your experimental goals and sample requirements in mind. This Single Cell Buyer's Guide can help you with that investigation. You can also leverage our single cell product selector to find the tool that best fits your needs.

2. How can I run my 10x Genomics single cell assays?

Once you know what product is right for your biological questions, you will also want to consider throughput and scale requirements. This will guide you to the appropriate instrument platform by which you will run the assay.

Importantly, your experimental ambitions may start in one place, only to grow and evolve to a new scope you may not have originally anticipated. Flexibility in throughput and scale to match your goals is a huge value that the Chromium Single Cell platform from 10x Genomics embodies. Our instrumentation includes the Chromium X Series, Connect, and Controller, each employing advanced microfluidics to perform single cell partitioning and barcoding in a matter of minutes.



Chromium X Series	Chromium Connect	Chromium Controller
The next-generation single cell instrument, providing the ultimate flexibility to conduct experiments at the scale you need.	The automated solution for your single cell sequencing workflow, letting you go from cells to sequencing-ready libraries with walk-away convenience.	The classic single cell instrument enabled for low and standard throughput solutions.
Learn more about the Chromium X Series	Learn more about the Chromium Connect	Learn more about the Chromium Controller

The flexibility of this instrumentation is matched by our low- and high-throughput Single Cell Gene Expression kits, enabling everything from pilot experiments to comprehensive translational studies.

Explore your throughput options with this interactive map, profiling the Chromium X Series.

Map your single cell journey ⟶

If you're not in a position to purchase your own Chromium instrument, there are other options for you to run your single cell assay. For example, your institution may have genomics core labs with available Chromium instruments, or you may be able to share resources with other labs. Additionally, we have a number of Certified Service Providers trained on 10x Genomics best practices, giving you access to the expertise of other scientists and bioinformaticians to support your research. You can search for Service Providers in your region using this database.

Frequently asked questions

3. Do I need to be a bioinformatician to analyze sequencing data derived from a 10x Genomics single cell experiment?

No, you do not need the background or skills of a bioinformatician to analyze your data. That's because single cell solutions from 10x Genomics come with intuitive software for data analysis and visualization. Cell Ranger is our suite of analysis pipelines that turn your raw sequencing data into results. This analysis software can be used to generate expression profiles for each cell, identify clusters of cells with similar profiles, and aggregate data from multiple samples.

The output of Cell Ranger pipelines includes QC information and files that can be easily used in our Loupe Browser visualization software. Loupe is a point-and-click desktop software that's easy for anyone to download and use, and enables you to interactively explore your results. This includes the ability to filter out certain cells, recluster your data, study differential expression patterns for genes of interest across cell clusters and samples, determine cell types, and chart results in violin and heatmap plots.

10x Genomics Cloud Analysis can streamline this process even further, allowing you to run Cell Ranger analysis pipelines with a simple web interface and scalable infrastructure for fast results. Regardless of whether you are analyzing one library or one thousand libraries, each 10x Genomics dataset can be stored on the platform for 90 days and used in 5 of our analysis pipelines.[1] Cloud Analysis is currently available in the US only, but anyone in the world can freely download both Cell Ranger and Loupe Browser from our support site and analyze 10x Genomics datasets on their own computing environment.

Cell Ranger produces standard format output files that can be used with a variety of third-party tools. Some popular tools for gene and protein expression data analysis include Seurat, Scanpy, and Bioconductor. These tools typically come with tutorials that can help familiarize you with how they work.

Finally, specific data analysis questions can always be directed to the expert 10x Genomics Software Support team at support@10xgenomics.com.

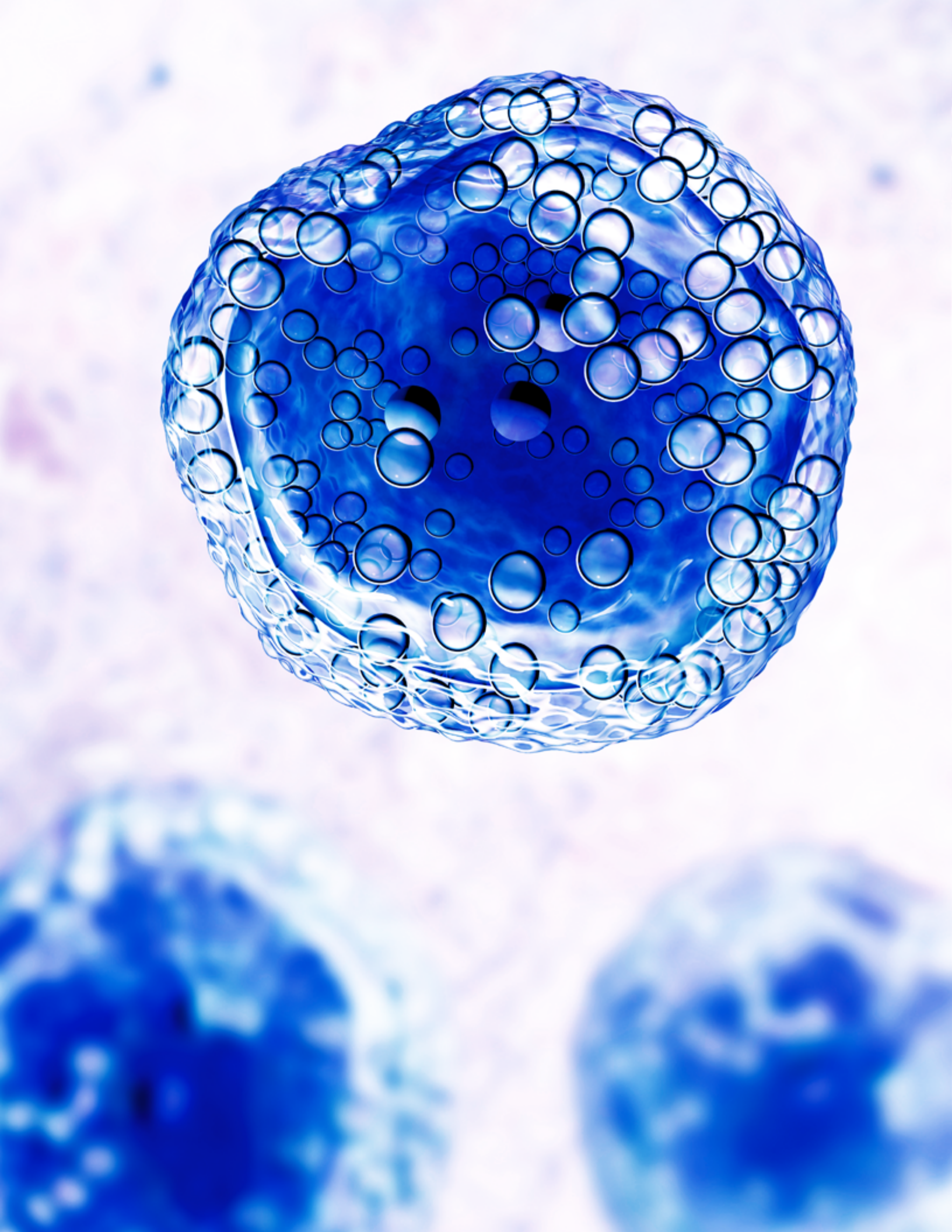
*1 See 10x Genomics Cloud Terms of Use for restrictions and details.

4. Are there additional resources that I can prepare with?

Yes, plenty! Once you're ready to begin preparing for single cell experiments, this catalog of 10x Genomics User Guides can help you choose the appropriate protocol for your assay. Additionally, this How-To Video Series is incredibly helpful for getting a feel for the workflow, including wet lab techniques that you'll need to be proficient in and important stopping points.

Finally, we offer grant application resources for some of our single cell products that can support you as you seek funding and approval of planned single cell experiments:

- Chromium Single Cell Gene Expression (including low-throughput kits)
- Chromium Single Cell Multiome ATAC + Gene Expression



Conclusion

Changing what "possible" looks like

"They have been out of our reach—now we have reached in. Whether you are studying rheumatoid arthritis, diabetes or the brain, you have the chance to ask each cell what it is doing."

- Dr. Francis Collins, Director of the National Institutes of Health (1)

"The innovations change what's possible. So the Human Cell Atlas wouldn't have been feasible to even think about 20 years ago."

- Dr. Sarah Teichmann, Head of Cellular Genetics, Co-Founder of the Human Cell Atlas, Senior Group Leader, Wellcome Sanger Institute (2)

The way we do science is fundamentally changing. Now it is possible for us to interrogate each single cell in a complex sample, to understand its identity and function in the larger system, and to resolve poorly characterized or hidden cellular and molecular mechanisms at work in health and disease. Traditional methods of transcriptomic interrogation have enabled many crucial discoveries, but the complexity of biology requires ever deeper investigation, ever increasing resolution and refinement.

In this eBook, we've demonstrated the new depth of insights that are possible with single cell sequencing technology. With so much more information-rich data from your samples within reach, you can be confident that your next breakthrough—the next generation of discoveries that have the potential to transform how we understand and approach cancer, autoimmune disorders, vaccination efficacy, neurodegenerative disease, and more—will come at single cell resolution.

References

1. University of Alabama at Birmingham. Looking to the future with Dr. Francis Collins. March 6, 2020. https://www.newswise.com/articles/looking-to-the-future-with-dr-francis-collins.
2. 10x Genomics. Science of Tomorrow. https://www.10xgenomics.com/science-of-tomorrow/.

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Single cell solutions from 10x Genomics

Chromium Single Cell Gene Expression

Analyze gene expression, surface protein expression, and CRISPR perturbations in single cells.

- Capture the full heterogeneity of a sample to characterize complex cell populations, discover novel cell types and states, and identify biomarkers.
- Choose the experimental throughput that fits your needs, starting with small-scale pilot experiments to large-scale translational studies.

Learn more ⟶

Chromium Single Cell Multiome ATAC + Gene Expression

Analyze chromatin accessibility and gene expression simultaneously in single cells.

- Enhance your characterization of cell types and states, and reconstruct cell type–specific gene regulatory programs driving cellular lineage and differentiation.
- Identify target genes and cell types of genetic variants associated with disease.

Learn more ⟶

Targeted Single Cell Gene Expression

Capture expression profiles of a set of targeted genes from hundreds to thousands of single cells.

- Scale your studies, refine discoveries, and validate biomarkers and drug targets by focusing on the genes most relevant to your research.
- Profile a defined set of transcripts with customizable, pre-designed human panels for cancer, immunology, neuroscience, and drug discovery, or design a fully custom panel with up to 1,500 gene targets.

Learn more ⟶

Chromium Single Cell Immune Profiling

Analyze full length paired B-cell or T-cell receptors, surface protein expression, antigen specificity, and gene expression all from a single cell.

- Use single cell multiomics to discover new biomarkers, identify novel cell types and functions, and recover a comprehensive immune repertoire.
- Perform antibody discovery and immune receptor mapping, or combine it all together.

Learn more ⟶

Chromium Single Cell ATAC

Analyze chromatin accessibility at the single cell level.

- Profile the chromatin landscape cell by cell and identify transcription factor binding sites in tens of thousands of cells at single cell resolution.
- Gain a deeper understanding of gene regulatory mechanisms to explore the epigenetic underpinnings of disease, developmental plasticity, and cell identity.

Learn more ⟶

Resources

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